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# STUDIES ON AUTO-DIGESTION

## I. The Fate of Organs Implanted into the Duodenum[1]

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The following work was undertaken in order to find under what conditions organs implanted into the duodenum or upper jejunum might be digested.

It is well known, especially from the surgical literature, [see Rost (1923) v. Lindhardt (1925)] that diseases or traumata of the pancreas may occur, in which active ferments of the pancreas are found in the peritoneal cavity Under these conditions lipolytic and tryptic digestion [Truhardt (1902), v. Bergmann (1906), Seidel (1913)], may be observed not only in the abdomen, but also in other parts of the body, e.g., pericardial fat [Langerhans (1890)]. This seems to be due to emboli, [Eppinger (1905), Payr u. Martina (1906)]. We assumed that by some factor (occurring simultaneously or consequent to the disease or trauma of the pancreas), the resistance of living tissues to disgestion might be decreased. We therefore implanted organs into the duodenum, thus imitating the conditions under which digestion occurs in the abdomen, etc., when the pancreas is injured. In both cases living tissue is exposed to the action of the active ferments of the pancreas. We then tried to influence the resistance of these implants against digestion.

Only one previous study on the implantation of organs in the duodenum occurs in the literature. Rosenbach (1911) implanted the *cauda pancreatis* of a dog into its lower duodenum and noted, that if the blood supply of the pancreas was not injured and the organ not roughly handled, no digestion occurred. As the pancreas might have a special resistance against its own ferments, we used mainly omentum implants in our experiments.

[1] A preliminary report was read before the Sixth Congress of the Far Eastern Association of Tropical Medicine at Tokyo, Japan, October, 1925.

TECHNIQUE. The entire investigation was carried out in dogs. We implanted a lobe of the liver, the upper pole of a kidney and pieces of the omentum into the intestine.

For the liver and kidney, the upper jejunum was taken, for the omentum, the lower part of the duodenum.

The serosa was peeled off the liver and kidney at the place where they were opposed to an incision on the anti-mesenterial border of the intestine. They were fixed to the latter by a few stitches, the junction between organ and intestinal wall being then sealed by suturing a portion of omentum to both the organ and the serosa-muscularis of the intestine.

The technique for implanting the omentum was as follows: An incision of about 1 cm. in length was made on the anti-mesenterial border of the distal duodenum or proximal jejunum; the tip of a piece of omentum was tied with silk ligatures, the ends of which were left about 10 cm. long. These were next threaded on long straight needles which were passed caudally through the opening in the intestine for about 4 cm. when they were pushed outwards through the intestinal wall. By pulling on the silk a long piece of omentum was drawn into the intestinal lumen, and its tip fixed by a stitch of the silk. The opening in the intestine was closed by suturing the external omentum to the serosa of the intestine. This gave a good closure in a very short time. At various intervals after the first operation the intestine was again opened to observe the condition of the implant.

EXPERIMENTAL RESULTS. The dogs were observed from 3 hours to 73 days after implantation, the state of the implant being ascertained at laparotomies performed at different intervals between these two periods. The implants were so strongly anchored to the intestine, that they could not possibly slip back into the peritoneal cavity. As the result of peristalsis or local intestinal spasm or of spasm of the blood vessels of the implant or in consequence of undue pressure being exerted by the necessary fixation of the root of the implant to the serosa of the intestine, the blood supply of the implant may have suffered in some animals more than others. This may have occurred in those control animals, where we found that the implant had disappeared or had diminished to only a few millimeters. The cause of the disappearance or diminution of the implant would thus be due to digestion of tissue rendered abnormal by a deficient blood supply; this point, however, will be discussed later.

In 2 of the 45 observations the implant was found to be absent, 15 and 32 days respectively after the original implantation. In 5 of the dogs the implant was "very small" after 3 to 11 days. Ten dogs in which the implant had been found to be present 6 to 15 days after implantation were subjected to a second laparatomy 3 to 32 days after the first control-laparatomy; in all ten the implants were found to have persisted.

In another series [Necheles and Fernando (1925b) tables 2 and 3], 13 further observations on omentum implants (from 1 to 27 days) are recorded; one implant in this series was found to be very small (after 27 days). Thus out of a total of 58 observations, the implants were found to have disappeared entirely in only 2 experiments (3.5 per cent) while a marked diminution of the implants was found in 6 observations, making a total of 8 in all (13.8 per cent).

Many of our dogs died from diseases caused by the worm infections so frequently found in Peking, by distemper and by other purulent processes.

Mention must be made of the state of the implants themselves. Soon after the implantation it was found that some of the omental

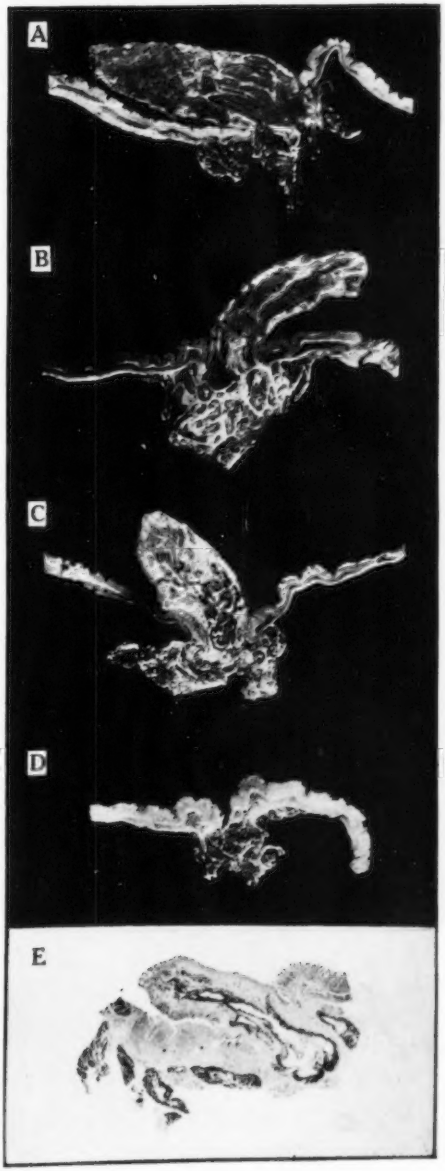


Fig. 1. Photographs of omental implantations.

The implant, along with the adjacent intestine has been sectioned longitudinally, the mucosal surface being placed uppermost. A, B, C, D, × ¾, stained Sudan III; E × 1.7, stained osmic acid; the fat appears black. A = dog 182, 5 days, tip of implant hemorrhagic, due to suture; B = dog 187, 18 days; C = dog 59, 73 days, old hemorrhage at tip due to suture; D = dog 159, implant absent, 8 days after treatment with pancreas; E = dog 1, 9 days

implants were edematous. This edema disappeared later, and adhesions took place between the layers of omentum composing the implant, thus producing a solid cone. In the outer layers of this cone a slight process of inflammation was observed microscopically. Finally a slight contraction of the cone (recognized as such by the position of silk knots in the implant) takes place (see fig. 1). Rosenbach (1911) observed similar changes in the implanted pancreas. The most remarkable fact was that the hemorrhagic (peritonitis) inflammation, which frequently occurs when trypsin [Trypsin Merck, or Trypsin prepared by R. Heidenhain's (1875) method or duodenal juice (from a dog with a Pavlov-Kestner duodenal fistula) or fresh pancreas (dog)] is administered intraperitoneally [v. Bergmann (1906)] or after the production of artificial necrosis of the pancreas, was never observed in the omentum implants.

Rosenbach (1911) placed trypsin solutions on the tongue of living frogs and observed vasodilatation, diapedesis and hemorrhages under the microscope; controls with inactivated solutions had not the same effect. The cause of the vascular stasis and hemorrhages is believed by some authors to be due to a toxin and by others to arise from the activity of the trypsin [see v. Bergmann (1906), Polya (1908) and Migay and Petroff (1923) and others]. Our observations clearly demonstrate the fact that healthy living tissue, implanted into the duodenum or upper jejunum is not digested.

The starting point for our work was the phenomenon that under certain pathological conditions (as mentioned above) digestion of living tissue occurs. Digestion of fat occurs in the disease "fat necrosis;" while after the injection of pancreatic juice or inoculation of the whole pancreas, we have observed lipolytic and tryptic digestion in the peritoneal cavity of dogs. We imitated these conditions in our implantation-experiments, but no evidence of digestion could be obtained. In consequence of the fact that trypsin solutions are said to have a detrimental action on blood vessels, we expected to find at least some sign of this influence on the omental implants, which we controlled for this purpose from 3 hours to 73 days. As, however, we never observed a hemorrhagic inflammation of the implant, this might be explained by its relatively small surface. Under the above mentioned conditions, where digestion occurs, it is possible, that the whole surface of the peritoneum absorbs ferments or toxic substances [Shigehiro (1924–25)], which, after being transported by the blood to the tissues, so act upon them, that they are rendered more liable to digestion. In order to produce the same conditions in animals with omental implants, we decided to create an opportunity for the absorption of ferments or poisons such as may be conceived to occur under pathological conditions of the pancreas (viz., fat necrosis).

*1. Injections of trypsin:* In 11 dogs the implant was found to have dis-

appeared in 3 animals (27.27 per cent) 9, 13 and 22 days after commencement of treatment. Compared with the experiments wherein more and longer observations (up to 73 days) were made, the number of implants which completely or partially decreased (15.5 per cent) shows clearly that a difference exists between treated and untreated animals.

*II. Inoculation of pancreatic tissue:* The next series of experiments was carried out by inoculating the fresh sterile pancreas of dogs into the peritoneal cavities of animals with omental implantations. It was supposed that autolysis of the pancreatic tissue would occur and that active ferments and poisonous substances arising from the necrotic process would be absorbed through the peritoneum.

In 2 of the above experiments the implant was found to be absent (22 days after the commencement of inoculation, when a control laparotomy had shown the implant to be present). In the other two dogs the implant was present 3 and 11 days after the last control laparotomy. Thus we see that out of 4 experiments, 2 were found to be negative after the treatment (i.e., 50 per cent against 15.5 per cent of the controls).

*III. Artificial necrosis of the pancreas:* In order to create a state as near as possible to the conditions under which pathological digestion of living tissue occurs, we induced artificial necrosis of the pancreas by the injection of sterile olive oil into the main duct [Bernard (1850)], which was then ligatured.

Of the implants in 5 dogs with necrosis of the pancreas, 2 were found to have disappeared on the day after the onset of necrosis, while one was in a state of hemorrhagic inflammation and semi-digestion after the same interval, a state which we never observed in the ordinary omental implantations (40 per cent or 60 per cent compared with 15.5 per cent).

It is interesting to note that in most of these experiments the mucosa of the whole stomach and small intestine was found in a state of marked hemorrhagic inflammation. Since injection of oil into the main duct only injures about three-fourths of the gland, while the remainder of the gland supplying the other duct remains intact, a small secretion may still occur into the lumen of the intestine. This might possibly be insufficient for the neutralization of the stomach juice; as, however, ulcers but no general irritation of the intestine are reported in cases in which the neutralization of the HCl of the stomach is eliminated [Dott and Lim (1923) and others], this cannot explain our findings. One might suppose therefore, that in the above cases the resistance of the mucosa of the whole intestinal tract against digestion had suffered.

*IV. Parathyroidectomy:* According to some recent publications evidence has been brought forward, that in parathyroidectomy the permeability of the intestine becomes changed, permitting poisons and perhaps ferments to pass into the blood from the intestine. Spadolini (1924) found

edema, hemorrhage and necrosis of the mucosa of the intestine after parathyroidectomy.[2] We have employed this procedure on our implanted dogs with the view of increasing the permeability of the intestine.

As guanidines are made responsible, by various authors, for the symptom-complex of parathyroidectomy, we also treated one dog with injections of methyl-guanidine chlorhydrate [Shanks (1923–24), Luckhardt and Compers (1923–24) Dragstedt (1924), Jacobsohn (1924), Sinelnikoff (1925), Herxheimer (1925)].

As it has been shown again recently by Migay and Petroff (1923), that croton oil changes the permeability of the intestine (in which event it might have some irritating effect on the implant itself), we also subjected one dog with an omental implant to a prolonged treatment, giving the oil *per os* in daily increasing amounts.

Of 11 dogs subjected to thyro-parathyroidectomy or parathyroidectomy only 2 were found to have lost their implants. The treatment with methyl-guanidine and croton oil had likewise no effect on the fate of the implant (15.3 per cent). Compared with the control experiments (15.5 per cent) no change in the fate of the implants was thus effected.

Discussion. The result of our experiments on dogs with implants in the duodenum and upper jejunum shows that the implants are resistant against digestion. This makes it evident that neither the assumed specific resistance of the epithelium of the intestine, nor its mucous covering are the real protectors against digestion, as they are absent in the case of the surviving implants. Another assumption [Bayliss (1919)], that the free enzymes are adsorbed partly by mucus and partly by food, and that the alkalinity of the pancreatic juice is decreased or neutralized by the stomach juice, seems to us improbable, as we nearly always find active ferments in an alkaline medium in some portion of the small intestine in starving animals. The stomach juice in a starving animal is generally neutralized in the upper part of the jejunum.

We have done some work on the question of antitrypsin but found that it is probably not the protector of the implants [see Necheles et al. (1925a, b)].

It is possible that both ferment and alkali of the pancreatic juice may, after quick absorption by the intestine or implant become neutralized or inhibited so long as the circulation remains intact.

We have demonstrated, that it is neither possible to imitate the conditions obtaining in disease of the pancreas, nor in experimental conditions under which digestion of living tissue occurs, by simply exposing the omentum (implant) to the pancreatic juice within the intestinal cavity. We, therefore, thought that by lowering the permeability of the intestine

[2] In the autopsies of our dogs suffering from tetany, no macroscopic ulcers in the stomach or intestine could be detected.

so that ferments and toxins might be more readily absorbed, the resistance of the implants against digestion would be lowered also, whether the resistance be local or humoral (general). The experiments employing parathyroidectomy, methyl-guanidine, and croton oil were directed to this end, and although the animals died from intoxication and weakness (showing at least that body resistance in general was lowered), the absence of digestion of their implants indicated that the above factors have no influence. The observations on many dogs which died from various severe diseases, show that a lowering of body resistance alone has no influence on the fate of the implant.

Our experiments in which the implants disappeared to a greater extent than the controls, make it clear that the fate of the implants is dependent on some toxic principle of the pancreas itself. Further work must be carried out on these lines. Some previous observations point to high toxic compounds of the pancreas as being responsible for the sudden collapse in acute necrosis of that organ [Egdahl (1907), Polya (1908), v. Bergmann u. Gulecke (1910), Whipple (1914), etc.]. More definite evidence of the pancreatic principle, however, remains to be worked out.

The fact that the implants disappeared to a much greater extent in the pancreas and trypsin inoculation-experiments than in the controls, may perhaps be explained in the following way. The blood supply of the implants may not all be equal in consequence of local spasm of the intestine, of peristalsis, or of pressure by misplaced sutures. If now a toxin interferes with the blood supply or perhaps with the vitality of the tissue those implants will be digested whose blood supply is already impaired. We have no evidence, however, that strong spasms of the intestine occur after intraperitoneal treatment with pancreatic juice, etc.; on the contrary, the hemorrhagic peritonitis indicates an atony of the intestine.

## SUMMARY

1. Kidney, liver and omentum implanted into the duodenum or upper jejunum of dogs remain in most cases undigested during a period of observation extending from 3 hours to 73 days.

2. Intraperitoneal injections of trypsin solutions, inoculation of fresh sterile pancreas and artificial necrosis of the pancreas itself seem to facilitate the digestion of a number of omental implants.

3. Parathyroidectomy, subcutaneous injection of methyl-guanidine and croton oil *per os* do not influence the fate of the implants.

4. It seems probable that certain toxins of the pancreas are necessary to induce digestion of living tissue. This may explain the occurrence of digestion in necrosis of the pancreas, after intraperitoneal injection of pancreatic juice and inoculation of pancreatic tissue.

We wish to express our thanks to Dr. R. K. S. Lim for his valuable advice and criticism in the course of this work.

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# STUDIES ON AUTO-DIGESTION

## II. The Rôle of Trypsin and "Antitrypsin"[1]

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The mechanism by which the intestine is protected against digestion by its own ferments is not known; according to many authors the so-called "antitrypsin" of the serum plays an important rôle [Hermann (1881), Frenzel (1891), Weinland (1903), Pribram (1909), Kolmer (1917) and Tigerstedt (1919) and others]. Many experiments have been published, which appear to show that the "antitryptic" power of the serum is increased by intraperitoneal and subcutaneous injections of trypsin or trypsin-like ferments (goats, dogs, rabbits, guinea pigs and geese) [v. Dungern (1898), Dean (1901), Sachs (1902), Hahn (1903), Opie (1905), Jochmann u. Kantorowitz (1908), Jochmann u. Lockemann (1908), Bergmann u. Bamberg (1908)]. Changes of the "antitryptic" titre have also been recorded after feeding pancreatin *per os* [*pro et contra* Bergmann u. Meyer (1908), Brieger u. Trebing (1908), Meyer (1908)]. Other authors, however, found it impossible, or at least exceedingly difficult to provoke any change of the "antitryptic" titre by the above mentioned means [Landsteiner (1900), Bergell u. Schütze (1905), Döblin (1909), Rosenthal (1910), Bauer (1910), Kaemmerer u. Mogulesko (1911–12), Young (1918), Wertheimer (1923)]. Much work has further been done to increase the "antitryptic" titre of the serum by means of drugs; thus uranium and chromium salts, phosphorus, corrosive sublimate, iodine, chloroform, phloridizin, thyroidin, etc., have been tried but the results have contradicted one another [Braunstein (1909), Meyer (1909), Cobliner (1910), Hirata (1910), Jobling and Petersen (1914), etc].

The "antitrypsin" of the serum has been associated with the following constituents of the blood by various authors: globulin [Glaessner (1904), Sugimoto (1913) (egg-globulin)], albumin [Cathcart (1904), Hedin (1904 and 1906–7), Opie and Barker (1907), Kaemmerer u. Mogulesko (1911–12)] lipoids or lipoid-protein compounds [Schwartz (1909)]; compounds of

[1] A preliminary report was given at the IVth Congress of the Japanese Physiological Society, Mukden, China, August, 1925.

unsaturated fatty acids [Pribram (1909), Schwartz (1909), Jobling and Petersen (1914) Opie (1922), etc.], unknown colloidal substances [Döblin (1909)], and protein cleavage products [Rosenthal (1910–11), see Bayliss (1919) for contrary view]. Excretion of a tryptic ferment in the colostrum, which would automatically increase the "antitryptic" titre in the serum [Jochmann u. Mueller (1906), Graefenberg (1909)], may be also mentioned.

It should also be stated that there is no lack of denials or counterproof to each of the above contentions [Rondoni (1910), etc.]. Thus in diseases with augmented lipoid content of the serum the "antitryptic" titre may be normal or even decreased [Peritz (1908), Meyer (1909), Cobliner (1910), Stümpke (1910), Meyer (1911), Stawraky (1914)]. Pribram (1909, p. 84) claims (in accordance with results of Hoeber, Porges and Neubauer) that lipoids are simultaneously precipitated by the usual methods of precipitating albumin and globulin.

The experiments of Plaut (1914), Jobling and Petersen (1914), Jobling, Petersen and Eggstern (1915–1915a) based on those of Hedin (1904), Pribram and Pick (1909), Wassermann u. Keysser (1911), etc., give evidence that the "antitryptic" titre is the product of two components; one a proteolytic ferment and the other an "antitryptic" substance. The former may be set free by adsorption of the latter on kaolin, barium sulfate, starch, kieselguhr, or by means of iodine, chloroform, etc. This ferment has been termed protease by Jobling; it acts in either a weakly alkaline or a weakly acid medium. In the latter the action is apparently much weaker [Eggstein (1914)] than in the former. There is, however, some evidence [Opie and Barker (1907)], that the above properties of protease are in reality due to the existence of two different proteolytic ferments in the serum, the one a pepsin-like ferment and the other a trypsin-like ferment (they are possibly proferments).

The protease acting in an alkaline medium is inhibited by the "antitrypsin" in the same way as trypsin is inhibited. Thus we see that if attempts are made to isolate "antitrypsin" the possibility arises that the protease may be damaged or destroyed, resulting in an apparent increase of the "antitryptic" power. This may have occurred in Schwartz' experiments (1909); which were intended to prove that lipoids of the serum contain the "antitryptic" factor. He stated that lipoids could be added and thoroughly mixed with normal serum, without causing any change of the "antitryptic" titre; if, however, the mixture was heated one hour at 65°C., a significant increase of the "antitryptic" titre was obtained. It is apparent that this procedure, by destroying protease, may suffice to cause the increase of the "antitryptic" titre.

Similar conditions may have prevailed in some of the other experiments carried out to separate and determine "antitrypsin." According to Opie and Barker (1907) the "antitrypsin" in the serum is destroyed by a tem-

perature of 75°C., but is unaffected by heating to 70°C. for half an hour. Different values, however, are given by other authors, and it seems as if there are several factors determining the rate of destruction of "antitrypsin" as well as trypsin at various temperatures. [Chittenden and Cummins (1885), Cathcart (1904), Kaemmerer u. Mogulesko (1911–12)]. Jobling, Eggstein and Petersen (1915) have noted that dog's serum contains little or no protease; in another work (1915a) they state, that after feeding there is a progressive decrease in serum protease of dogs. There must therefore be a physiological variation in the concentration of the ferment which might explain the so-called variation in the "antitryptic" titre.

According to Jobling, Eggstern and Petersen (1915), etc., heating of the guinea pig's serum at 56°C. for 30 minutes, causes a marked impairment of the protease activity, and heating at 56°C. for a period longer than 30 minutes or at 70°C. for half an hour, destroys the protease altogether.

There seems to be a general consensus of opinion, that antibodies against ferments cannot be built at all, and that "antitrypsin" is not a specific antibody [Meyer (1909), Meyer (1911), Bayliss (1919, 1922), Opie (1922)].

Stawraky (1914), Citron (1922) and others, however, maintain that "antitrypsin" is a true antiferment.

One frequently finds the statement that the "antitryptic" power of the serum may be due to unspecific bodies; that is to say certain components of the serum may be increased or decreased under the influence of trypsin-like ferments of which three are believed to be more or less important; Opie's leucoprotease [Opie and Barker (1907)], found in leucocytes, [Wiens (1907a), Jochmann (1908)], the tryptic ferments found in cells in general [Aronsohn (ferment of the embryonic chorion) (1906), Graefenberg (1909), Meyer (1909), Meyer (1911)], and the trypsin of the pancreas [Jochmann u. Lockemann (1908), Cobliner (1910)]. These ferments are supposed to enter the blood in small amounts, and to stimulate the production of unspecific antibodies which hinder the action of any of these different ferments [Jochmann u. Kantorowitz (1908), Graefenberg (1909), Opie (1923)]. If these ferments enter the blood in very large amounts, however, the "antitryptic" titre is at first neutralized, but after this initial decrease a reactive increase takes place.

If active pancreatic juice is injected intraperitoneally in big doses, it nearly always causes death within a few hours (1 to 6 hours); in most cases there is a hemorrhagic fibrinous exudation and, depending on the pancreatic preparation used, fat necrosis or signs of tryptic digestion. This is especially the case, if the sterile pancreas itself is administered intraperitoneally [see also Gulecke (1903), v. Bergmann (1906), Necheles (1925c)].

Subcutaneous injection of active trypsin produces marked local ulcers which persist for a long time. If, however, an animal is injected intraperitoneally once or twice with small doses of trypsin, it may be treated

later with much larger, and even lethal doses, by either the intraperitoneal or the subcutaneous route without harmful effect. This resistance was ascribed by Achalme (1901) to an immunization against trypsin. As Bergmann and Gulecke (1910), however, succeeded in immunizing dogs with trypsin heated to 70°C., at which temperature the ferment is destroyed they concluded that immunization against a toxic substance contained in the trypsin solution had taken place. This view is sustained by the experiments of Young (1918) who showed, that a precipitin was present in the serum of animals treated with injections of trypsin solutions.

It must be mentioned here, that some authors found no harm to follow injection of active trypsin, either intraperitoneally or intravenously, provided it was previously purified as much as possible [Fermi and Pernossi (1894), Schittenhelm u. Weichardt (1911), etc.], and also sterilized (without destroying its activity). We believe, that intoxication always happens, if digestion of living tissues occurs.

The aim of our own experiments was to study whether the resistance of organs implanted into the duodenum below the orifices of the pancreatic ducts against tryptic digestion was due to the "antitrypsin" of the serum. The result of these experiments [see Necheles, Ling and Fernando (1925b)] made it necessary to extend the investigation to the question of the relationship between trypsin and trypsin-like ferments, and "antitrypsin." We have implanted a lobe of the liver and a part of a kidney into the duodenum; in the first dog after 24 days, in the second dog after 8 days, the organs were found uninjured in the intestine. It may be noted here, that Rosenbach (1910–11) implanted the pancreas itself into the duodenum and found that if the blood supply remained uninjured, and the organ was not handled roughly, no signs of digestion occurred.

All other experiments were carried on with omentum implanted into the duodenum. The reason for this was the following: It has been shown [Katsura (1924), etc.] that the omental lymph channels may absorb besides liquids also small corpuscular elements from the abdominal cavity. As some authors have found an increase of the "antitryptic" titre of the blood after intraperitoneal injections of trypsin solutions, we imagined that omental implantations would permit absorption of trypsin into the blood and thus provoke a reactive increase of the "antitryptic" bodies, assuming that the normal "antitryptic" content of the blood is insufficient for the protection of the implants. Thus it follows that the implant will be protected to a higher degree against tryptic digestion. This idea was also suggested by the fact that the hemorrhagic inflammation which occurs in the intact omentum after intraperitoneal injection of trypsin, was never observed in the omentum implant; although the implant is equally exposed to tryptic action. Sometimes a simple edema of the implant could be observed within the first few days after implantation, but nothing more.

EXPERIMENTAL RESULTS. The "antitryptic" titre was determined by Bergmann's modification of the Gross-Fuld method[2] [Gross (1907–8), Fuld (1907–8), Bergmann u. Meyer (1908); for other methods see Hedin (1905), Mueller u. Jochmann (1906), Young (1918)]. We express the "antitryptic" titre as the difference between the smallest amount of trypsin[3] (0.1 per cent solution), which fails to digest a constant quantity of casein (2 cc. 0.2 per cent) in a constant time (20 minutes) at 37°C. with and without the addition of 0.5 cc. of serum (2 per cent dilution); if the "antitryptic" power of the serum was so great, that we had to employ higher serum dilutions, the above mentioned difference was multiplied by the degree of serum dilution employed. By this method we obtained constant values in spite of small variations in the strength of the trypsin and casein solutions.

We did not attempt to use Jacobs [see Braunstein and Kepinow (1910)] or Wohlgemuth's (1908) or Bergmann and Meyer's (1908) methods of calculation, as they appeared to us to be unnecessarily complicated. The accuracy of the Bergmann method has been ascertained by us to be within 0.1 unit of the "antitryptic" titre. It is not an end-point method as Young (1918) objects.

The "antitryptic" titre of normal dog's serum was found in 36 observations on 21 animals to be as follows; 37 per cent = 0.2, 48 per cent = 0.3, 6 per cent = 0.4, 3 per cent = 0.5 and 6 per cent = above 0.5 (in one observation a titre of 20.0 was found).

It is clear that the "antitryptic" titre in dogs serum is between 0.2 and 0.3, but in the same animal the "antitryptic" titre may increase without apparent reason. The high "antitryptic" titre in these dogs is not due to any of the parasitic worm infections, which commonly affect dogs in Peking, as we found the "antitryptic" titre to be normal in many dogs which on autopsy were found to be heavily infected.

Marcus (1908) working with Müller-Jochmann's method and using trypsin Kahlbaum (which as far as we know is prepared from pig's pancreas in about the same way as pancreatin Rhenania), finds that the normal human serum contains 4 units of "antitryptic" power. If we calculate the antitryptic power of the dog's serum from our own results, assuming that 0.3 is the average figure (note that we employed 2 per cent serum in our tests) we obtain a value of 3 units per cent of antitryptic power. Notwithstanding the difference of methods and errors of estimation there is a certain agreement in our numbers, especially as it is known that dogs' serum contains less "antitryptic" power than human serum.

It may here be mentioned that in one of our experiments, in which both

[2] On the apparatus used for determination of the "antitryptic" titre, see Necheles (1925a).

[3] We used one sample of pancreatin (*suillae, Rhenania*) throughout the entire work.

plasma and serum was examined, a very high "antitryptic" titre was found in the former, while in the latter the "antitryptic" titre was normal; this is confirmatory of the results of Marcus (1904); the "antitryptic" power of the plasma is probably due to fibrinogen and that of the serum to another substance.

Dogs 144, 159, 185, 186, 193 which were observed for 41, 12, 8, 20 and 8 days[4] respectively, remained completely unchanged or changed irregularly within narrow limits. As dog 158 had a high "antitryptic" titre before the implantation, the increase of the titre up to 1.0 after the operation cannot be ascribed to the operation or to the presence of the implant, especially as the titre fell after the implantation. If immunization had taken place, a steady increase should have been observed.

TABLE 1

*"Antitryptic" titre of dogs with omental implantation in duodenum*

| DOG | NUMBER OF DAYS OBSERVED | "ANTITRYPTIC" TITRE* | |
|---|---|---|---|
| | | Before | After implantation |
| 144 | 41 | 0.3 | 0.2, 0.2, 0.3, 0.3, 0.4 |
| 158 | 22 | 0.8 | 1.0, 0.4 |
| 159 | 22 | 0.5 | 0.3, 0.4 |
| 184 | 14 | | 0.5 |
| 185 | 8 | 0.2 | 0.3 |
| 186 | 20 | 0.2 | 0.2, 0.2 |
| 187 | 18 | 0.2 | 0.3, 0.5 |
| 190 | 7 | 0.4 | 1.0 |
| 193 | 8 | 0.3 | 0.3, 0.3 |
| 196 | 10 | | 0.4, 0.3 |
| 198 | 2 | 0.3 | 0.2 |

* In every dog a laparatomy was performed later and the intestine opened to inspect the condition of the implant (revision).

Dog 187 cannot be considered as normal, as he soon showed signs of distemper, followed by a fatal pneumonia, which perhaps explains the steady mounting of the "antitryptic" titre. It is well known that in human pneumonia the "antitryptic" titre may change very often (see later). Dog 198, although having a normal "antitryptic" titre should perhaps not be considered, as it died of peritonitis. Dog 196 showed signs of disease before the experiment and also when "revised." Thus only dog 190 remains, in which the "antitryptic" titre was increased after the implantation. The titre in this animal, however, returned completely to the normal (see table 2).

[4] After the implantation of omentum.

From these results we may conclude that in spite of any absorption of trypsin which may have occurred through the implant, there was no significant change of the "antitryptic" power of the blood. The question why the implants were not digested, although they did not possess the protecting epithelium and the mucous covering of the intestine, cannot, therefore, be answered satisfactorily by this result. While we may assume that the "antitryptic" power of the blood plays no rôle in the protection of the omental implant, it may be possible that the normal "antitryptic" power of the serum is sufficient without further increase, to protect the implant. (It should be noted that in duodenal ulcers the denuded muscularis is also not digested!)

The absence of any notable change in the "antitryptic" titre might be ascribed to a continuous fixation or saturation of any new "antitrypsin" which may be formed in response to trypsin absorbed. But the fact that the titre is so constant, is contrary to what might be expected if the above were true, e.g., if trypsin is introduced artificially into the blood, the "antitryptic" titre is said to fall (Jobling et al., 1915).

The idea has been brought forward lately, that in parathyroid-tetany the permeability of the intestine might be changed (perhaps in consequence of the Ca-deficiency of the tissues) and the symptoms of this disease might be provoked by poisonous substances passing abnormally into the blood from the intestine [Luckhardt and Compere (1923–4), Spadolini (1924), Dragstedt (1924), Jacobsohn (1924–5), Sinelnikoff (1925)]. We thought, that if these poisons could pass through, trypsin or any other substances which may affect the "antitryptic" titre, might also pass through.

The experiments of Migay and Petroff (1923) bring further evidence for this assumption. They found that introduction of active trypsin solutions into the duodenum of dogs produces no change in the blood pressure but, if a lesion of the mucosa was previously made or if croton oil had been given, a prolonged fall of blood pressure took place, showing that a change in the permeability of the intestine was an important factor.

We have, therefore, treated dogs with croton oil, expecting that a change of intestinal permeability would take place. Other experiments, in which dogs were treated with methyl-guanidin are also given in table 2 in order to show, that such a severe intoxication as this, does not produce any change in the "antitryptic" titre [on the relation between this intoxication and tetany see Jacobsohn (1924), Herxheimer (1925)]. In a previous paper [Necheles, Ling and Fernando (1925b)] we have shown that these procedures do not influence the fate of omental implants within the intestine.

It will be seen from table 2 that parathyroid-tetany, intoxication with methyl-guanidin and croton oil do not change the "antitryptic" titre. If, therefore, parathyroid tetany and croton oil irritation have increased the permeability of the intestine to trypsin, any absorption of trypsin which may occur does not provoke an increase in the "antitryptic" titre.

As in no case were we able to induce changes in the "antitryptic" titre, notwithstanding the fact that the implanted omentum seems to offer a ready surface for trypsin absorption, and that the various procedures outlined above in table 2 have also no effect, we proceeded to determine whether dogs injected (subcutaneously and intraperitoneally) with known quantities of trypsin would show any response of the "antitryptic" titre.

We found in all animals which had received intraperitoneal injections of trypsin, that hemorrhagic ascites almost immediately followed. Since it is known [Müller (1907), Jochmann u. Kantorowitz (1908a)], that ascitic fluid contains "antitryptic" properties, we decided to inject the trypsin subcutaneously in order to avoid fixation (neutralization) of the trypsin by the resulting ascitic fluid. Besides, nearly all our dogs normally possess a relatively large amount of peritoneal fluid, which might act in the above

TABLE 2

*Parathyroidectomy, methyl-guanidin, croton-oil*

| NUMBER OF DOG | PROCEDURE | NUMBER OF DAYS OBSERVED | "ANTITRYPTIC" TITRE | |
|---|---|---|---|---|
| | | | Before | After treatment |
| 144* | Twice 0.02 g.p.kgm. methylguanidin subcutaneously | 5 | 0.4 | 0 2 |
| 183 | Thyro-parathyroidectomy† | 14 | | 0.1 |
| 184* | Thyro-parathyroidectomy; 6 times 0.02 g.p.kgm. methyl-guanidin subcutaneously | 16 | 0.3 | 0.3, 0.2, 0.2 |
| 185* | Thyro-parathyroidectomy† | 7 | 0.2 | 0.3, 0.2 |
| 190* | Thyro-parathyroidectomy† | 8 | 1.0 | 0.3 |
| 193* | Thyro-parathyroidectomy† | 10 | 0.2 | 0.4, 0.4 |
| 196* | 1–3 drops croton-oil (stomach tube) | 5 | 0.3 | 0.3 |

* With omental implant.

† Tetany.

manner and this may possibly explain why certain dogs do not die after intraperitoneal injection of trypsin. The trypsin used was either Merck's preparation, or pancreatic juice (nearly bile free) obtained from a Pavlov-Kestner dog (metal cannula in duodenum, anastomosis between gall-bladder and jejunum, and the common duct ligatured).

Previous injections with trypsin solution heated to 70°C. or boiled, both being without tryptic power, also protected the animal against ulcers usually following subcutaneous injections of active trypsin solutions.

Dog 156 was treated with its own pancreatic juice in order to test whether autogenous trypsin differed in any way from heterogeneous trypsin. If autogenous trypsin had no influence on the "antitryptic" titre, it might explain why the dogs with implanted omentum did not show any

immunisation (it being assumed that the implant would present a ready surface for the absorption of the animal's own trypsin). Other workers have invariably injected heterogenous trypsin and the positive results obtained by them would thus be not inconsistent with our own observations.

The "antitryptic" titre in the above experiment (see table 3) remained unchanged in spite of repeated injections, so that thus far the hypothesis is tenable. We now proceeded to examine the effects of heterogeneous

TABLE 3

*Injections of trypsin*

| NUMBER DOG | PROCEDURE | NUMBER OF DAYS OBSERVED | "ANTITRYPTIC" TITRE | |
|---|---|---|---|---|
| | | | Before | After treatment |
| 156 | Subcutaneous injection of its own duodenal juice (3) and trypsin (2) solution | 37 | 0.3 | 0.1 (1*), 0.2 (3), 0.3 (7), 0.5 (1) |
| 177 | Subcutaneous and intraperitoneal injection of pancreas (1) extract and trypsin (2) solution | 25 | | 0.2 (1), 0.3 (2), 0.4 (1), 1.0 (1) |
| 207 | Subcutaneous injection of Aolan | 4 | 0.2 | 0.4 (2) |
| 208 | Subcutaneous and intraperitoneal injection of duodenal juice (3) and trypsin (2) solution | 36 | 0.3 | 0.2 (3), 0.3 (4), 0.4 (1) |
| 209 | Treatment as above | 36 | 0.3 | 0.2 (1), 0.3 (2), 0.4 (4), 0.5 (1) |
| 210 | Treatment as above | 36 | 0.4 | 0.2 (2), 0.3 (6), 0.4 (4), 0.5 (1) |
| 225 | Subcutaneous injection of duodenal juice (3) (large ulcer resulted). | 27 | 0.3 | 0.2 (2), 0.3 (2), 0.4 (1), 0.6 (1) |

(1) Sterile; prepared by Heidenhain's method; 3 cc. digest 3 mm. egg white (Mett tube) in 24 hours.

(2) Sterile trypsin (Merck) solution, 3 cc. digest 15 mm. egg white.

(3) From duodenal fistula (dog N. 156) 3 cc. digest 2 mm. egg white.

* The numbers in parentheses indicate the frequency of the observation.

trypsin for the purpose of comparison and to control the observations of other workers. Dogs 208, 209 and 210, were treated (see table 3) with homogeneous or heterogeneous trypsin over long periods, but they did not exhibit any change of the "antitryptic" titre which could be associated with a process of immunization. This is in contrast, for example, to the observation of v. Bergmann (1906), that a single large dose of trypsin produces a marked change of the "antitryptic" titre.

Dogs 177 and 255 showed a transitory but marked increase of the "antitryptic" titre which for the following reasons, we consider to be due to intoxication rather than immunization to trypsin. In the case of dog 177 active trypsin solution (Merck) had been injected daily intraperitoneally for five days, at the end of which period its "antitryptic" titre was 0.2; after receiving one injection of Heidenhain's trypsin solution, the titre rose to 1.0. Then the titre fell (0.3, 0.4) but increased to 0.6 after a laparotomy had been carried out to determine the state of the original implant. This dog had been very sick from the time the "antitryptic" titre had risen to 1.0 and subsequently died from a purulent peritonitis. In dog 225, the "antitryptic" titre rose at the same time the dog fell sick, but as the animal recovered and in spite of continued injections of Merck's trypsin the "antitryptic" titre returned to normal.

TABLE 4

*Dogs treated with pancreatic tissue*

| NUMBER OF DOG | PROCEDURE | NUMBER OF DAYS OBSERVED | "ANTITRYPTIC" TITRE (AFTER TREATMENT) |
|---|---|---|---|
| 158* | Dog's pancreas digested in trypsin solution† (24 hours) injected intraperitoneally; subcutaneous injection of trypsin† solution | 24 | 0.3, 0.3, 0.3, 0.2 |
| 159* | Dog's pancreas, autolysed 24 hours injected intraperitoneally | 18 | 0.4, 0.2 |
| 183 | Same as above. Subcutaneous and intraperitoneal injection of trypsin† solution | 36 | 0.3, 0.2, 0.2, 0.3, 0.3 |
| 186* | Intraperitoneal inoculation of fresh dog's pancreas; intraperitoneal injections of trypsin† solution | 3 | 0.4, 0.2 |

* With omental implant.

† Sterile trypsin (Merck) solution; 3 cc. digest 15 mm. egg white.

We may, therefore, conclude that trypsin, whether autogenous or heterogeneous, does not produce an increase of the "antitryptic" titre and that such increases as may be observed are not associated with the trypsin injections, as the increase is not sustained in spite of continuing the trypsin administration.

While we found no immunization against the ferment trypsin, we have noted in our animals a distinct immunization against some toxic fraction which accompanies many trypsin preparations (e.g., the toxin responsible for local sloughing and ulceration). Our observations, therefore, confirm those of Gulecke (1903), v. Bergmann (1906), Doberauer (1906), Pozerski (1909), Young (1918), and others.

We had now to test the influence of the other factors which are supposed to regulate the "antitryptic" power of the serum; i.e., the cell ferments and the ferment leucoprotease. We brought the fresh sterile pancreas of one dog into the abdomen of another or injected sterile autolyzed pancreas into the peritoneal cavity. By these procedures a relatively large amount of active trypsin is produced [Heidenhain (1875)], and at the same time other cell ferments are liberated; the irritation thus set up also provokes an intensive aseptic peritonitis accompanied by the presence of large numbers of leucocytes which on disintegration, set free their ferments.

The experiments recorded in table 4 show that no change of the "antitryptic" titre could be produced by the single or combined action of trypsin, cell ferments, leucoprotease and bacterial (one dog with purulent peritonitis) ferments. They are thus in opposition to those of v. Bergmann and Bamberg (1908), Bauer (1910), Braunstein and Kepinow (1910), Halpern (1911), but in agreement with those of Meyer (1909). Further evidence of the absence of relationship between the leucocyte ferment and the "antitryptic" titre may be gathered from the work of Juergensen (1910), Rosenow and Färber (1914) and Jobling, Petersen and Eggstern (1915); the only report which asserts that leucocytosis is accompanied by an increase of the "antitryptic" titre is by Wiens and Schlecht (1909).

In the course of our work many dogs died of diseases involving some purulent process (pneumonia, peritonitis); in none was the "antitryptic" titre above 0.5, whereas in half the animals the titre was normal (0.2 to 0.3). This confirms the findings of others [Hahn (1903), Stawracki (1914)].

It would seem, therefore, that the intoxication of disease processes may sometimes raise the "antitryptic" titre a little above the average normal, although it must be remembered that even in normal animals, the "antitryptic" titre may show a transient but strong increase without any ascertainable reason.

There may be some doubt as to whether the slight increase of the "antitryptic" titre in some diseases is a true sign of immunization to trypsin, especially as such a small rise may be due to a lowering of the normal trypsin-like ferments of the blood under these conditions. But as Falls (1915) (employing the Abderhalden method) and Jobling, Petersen and Eggstern (1915) state that in many infectious diseases the trypsin-like ferments of the blood may be increased above normal, the rise of the "antitryptic" titre in disease can hardly be due to a diminution of the tryptic ferment of the blood.

Since we have already shown that the "antitryptic" titre is increased neither by trypsin itself, nor leucoprotease nor cell ferments, the slight rise observed in sick dogs must be due to some other factor, as yet unknown. In many human diseases, conflicting reports concerning the "antitryptic" titre have been published, although in such diseases as cancer, sarcoma

and hyperthyroidism, there appears to be a more consistent increase of the titre. In the light of our work on dogs, we are of the opinion that the increase in the latter conditions is not due to a reaction to ferments but to the unknown factor, as mentioned above.

We may conclude, therefore, that the protection of the omental implants and of the intestine against digestion by trypsin is not due to an immunization against trypsin. "Antitrypsin" does not apparently exist as antibody.

The preceding, however, does not exclude the possibility of protection against digestion by some inhibitory factor in the blood. Therefore the assumption of an immunization as necessary for autodigestion is unnecessary.

OTHER OBSERVATIONS BEARING ON THE RELATION BETWEEN TRYPSIN AND ANTITRYPSIN. *The "antitryptic" titre in leukemia:* Müller and Jochmann (1906), Jochmann and Kantorowitz (1908) have published cases, in which they found active tryptic ferment in the circulating blood [this must be distinguished from the normal trypsin-like ferments of the blood, which are non-active as they are bound to some other substance ("antitrypsin")] and no free "antitrypsin." If, therefore, protection against ferments depends upon the formation of an antibody, "antitrypsin," the absence of this antibody in leukemia should have permitted the trypsin in the blood and in the intestine to digest the organs in the body. There are no records in pathological literature that either the intestine or any other parts are digested in leukemia.

TABLE 5

*Antitryptic titre in bird's serum*

| ANIMAL | ANTITRYPTIC TITRE |
|---|---|
| Chicken | 0.3 |
| Pigeon | 0.2 |
| Duck | 0.4 |
| Goose | 0.3 |

*The "antitryptic" titre in birds:* In view of Opie and Barker's (1907) and Schultz and Chiarolanza's (1908) statements that the serum of birds exhibits minimal or no "antitryptic" power, the hypothesis of protection against digestion by means of "antitrypsin," seemed no longer tenable.

In order to confirm this, we examined the "antitryptic" titre in the following birds (see table 5) in the same way as we had done in the case of dogs.

The above table shows that the "antitryptic" titre of birds is not much different from that of the dog, so that no conclusions can be drawn.

*The "antitryptic" titre of earthworms:* The oft-quoted experiments of

Frentzel (1891) and those of Weinland (1902, 1902a and 1903) and Hamill (1905–6), which showed that pressed juice of some parasitic intestinal worms possess strong "antitryptic" properties, have been utilized as important evidence in favour of the protective action of "antitrypsin." No controls, however, were carried out by the authors in order to determine whether other worms not inhabiting the intestine possessed the same quality. We, therefore, made the following experiment.

Twenty-eight earthworms (= 148 grams) were ground up in a mortar with sand with addition of 200 cc. of distilled water; 80 cc. of physiological saline solution and a few cubic centimeters of chloroform were added later. The mixture was well shaken and kept on ice for 12 hours; after filtration a clear yellowish fluid was obtained; this gave (in 2 per cent dilution) with $HgCl_2$, as well as with nitric acid, a slight precipitate. When acidified with acetic acid after the addition of concentrated NaCl solution and boiling [see Kestner (1925)], it became only slightly opalescent, while the 2 per cent sera of dogs, whether possessing high "antitryptic" titre or not, showed copious, cloudy precipitates under the same conditions.

The "antitryptic" titre of a 0.2 per cent solution of this (already strongly diluted!) extract was found to be 0.2, so that the tissues of the earthworm also possess a very high degree of "antitryptic" power. The teleological aptness of the intestinal worms possessing "antitryptic" properties may thus be questioned, although Kestner (unpublished), etc., has pointed out that the dead (killed) ascaris is quickly digested in trypsin solution while the living organism survives, from which it might follow that during life a production of protecting substances occurs.

Pribram (1909), however, has shown, that such substances as the "antitrypsin" of ascaris may be of the nature of fatty acid and that after death the decomposition of the juice of the ascaris liberates further fatty acid and pari passu the "antitryptic" titre increases (this is seen clearly if the acidity of the juice is controlled). The "antitryptic" titre of our earthworm extract was constant during two weeks and its reaction was invariably neutral to litmus. At any rate the phenomenon in intestinal worms can no longer be regarded as a specific protection against digestion by trypsin.

*The survival of living cells in active trypsin solutions:* Claude Bernard's celebrated experiment, in which he placed the leg of a living frog into the stomach of a living dog and found that the frog's leg was ultimately digested, has long been held to show that foreign living tissue could not resist digestion. Matthes has carried out a somewhat similar experiment on frogs, using a solution of trypsin, but in this case, the frog was not digested. It is known that even erythrocytes and spermatozoa may be subjected to the action of strong trypsin solutions for considerable periods without being damaged [Matthes (1902), Kirchheim (1914–15)].

We have examined the effect of hemolyzed blood on the digestive power of trypsin—and found that the degree of hemolysis had, within very wide limits, no effect. (This is not necessarily in opposition to the work of Abderhalden, who brought forward evidence for proteolytic ferments in erythrocytes). Weinland (1903), on the contrary, states that washed and ground corpuscles of the pig exert an "antitryptic" power. He was unable to isolate any trypsin-inhibiting substance although it is not difficult to isolate such substances in other instances. The assumption is, therefore, that the ground organic material may itself inhibit tryptic activity by adsorbing the ferment. Consequently this experiment of Weinland does not necessarily constitute a proof that the erythrocytes contain "antitrypsin."

TABLE 6

| DOG | "ANTITRYPTIC" TITRE | NUMBER OF DROPS OF 2 PER CENT SERUM IN NORMAL SALINE SOLUTION |
|---|---|---|
| 159 | 0.5 | 68.3 |
| 159 | 0.4 | 65.3 |
| 158 | 0.8 | 70.0 |
| 158 | 1.0 | 70.5 |
| 190 | 0.3 | 69.0 |
| 124 | 0.2 | 68.3 |
| Pressed juice of earth-worms | Very high | 63.0 |

TABLE 7

| | CONSTANT CASEIN-TRYPSIN SAPONIN IN VARYING QUANTITIES | RESULT |
|---|---|---|
| Serum 1 | 1 cc. 1/1000 saponin | 0.5 |
| Serum 2 | 3 drops 1/1000 saponin | 0.5 |
| Serum 3 | 1 cc. 1/1000000 saponin | 0.5 |
| Serum 4 | Nil (control) | 0.5 |

The supporters of the "antitrypsin" hypothesis will regard Kestner's, etc., observations on ascaris as being fundamental. The interpretation of this fact, according to the above view, must be, that a continuous production of "antitryptic" substances takes place during life. The application of this reasoning to the erythrocytes, however, is hardly plausible.

*The question of focal immunity against trypsin:* Some authors [see Landsteiner's paper (1900)] have asserted that certain organs possess a focal immunity against trypsin on the basis of obtaining an "antitryptic" substance from extracts of organs, but Landsteiner (1900) has shown that after the blood had been washed out of the organs by perfusion, the "antitryptic" property is lost (except in muscle).

*What gives the "antitryptic" titre?* It was of interest to determine whether changes of surface tension could explain variations in the "antitryptic" titre; this seemed to us to be the most important physical factor which could exert an influence. Sera with normal and increased "antitryptic" titre were estimated with a Traube Stalagmometer. The results in table 6 show no clear relationship between "antitryptic titre" and surface tension.

A direct method was therefore used. To a standard series of trypsin-casein mixture saponin solution was added in varying amounts. The results were entirely negative, see table 7.

As the most probable physical hypothesis failed under experimental test to bring us nearer to the solution of our question, we give the following explanation, based largely on known experimental facts, for what it is worth.

It has been pointed out previously, that there is much evidence that the "antitryptic" titre is composed of 2 factors; an unspecific proteolytic ferment and a substance inhibiting it. Assuming this constitution of the "antitryptic" titre to be true, we may imagine the different possibilities of combination.

| | X | F | "ANTITRYPTIC" TITRE |
|---|---|---|---|
| I | 1 | 0 | = +(no ferment in blood) |
| II | 0 | 1 | = 0 (free ferment in blood) |
| III | 2 | 1 | = +(normal) |
| IV | 1 | 2 | = 0 (free ferment in blood) |
| V | 1 | 1 | = 0 |
| VI | 1 | >1 | = + |

X = Unit of trypsin inhibiting substance.
F = Unit of unspecific proteolytic ferment.

I. Examples of this are not known.

II and IV. To one of these belong the case of leucemia with free proteolytic ferment in the serum. The presence of the latter might be due to the absence of inhibitory substance or to the excess of ferment above the power of X.

III. Represents normal condition with inhibitory substance and bound ferment.

V. Is theoretically possible and has been reported to exist [Cobliner (1910) and others], but the methods employed were not sufficiently exact to be relied upon.

VI. Increase of the "antitryptic" titre with corresponding decrease of the ferment have been found in the course of digestion.

According to the results of our experiments we cannot agree with Cobliner (1910) and others who claim that this increase of the "antitryptic"

titre is caused by absorption of trypsin through the intestine during digestion resulting in a reactive production of "antitrypsin."

Cobliner (1910) and Stawraki (1914) found in depancreatized dogs, a steady decrease of the "antitryptic" titre which, however, never completely disappeared. This they regard to be a sign of the influence of absorbed trypsin acting as antigen.

As we have shown that trypsin does not act as antigen the decrease of the "antitryptic" titre cannot be explained in this way. If we consider the protease of the serum as a proteolytic proferment (see page 10), the absence of the pancreas results in an accumulation of ferment in the blood and consequently an automatic decrease of the "antitryptic" titre. The increase of the "antitryptic" titre during digestion may be more easily explained by diminution of the proteolytic ferment in the blood which is secreted into the digestive tract or stored in the emptied cells of the latter. This is in accordance with the experiments of Jobling, Petersen and Eggstern (1915) who found (in dogs) a decrease of the serum protease after a meal, and also with the observations of Cobliner (1910), that administration of HCl during a meal (children) increases the "antitryptic" titre still more (stimulation of the pancreas?).

A consideration of the work of others and the results of our experiments, show conclusively, that no antibody is formed against trypsin, but that certain substances (or certain physical states) may inhibit the activity of trypsin. It would be more correct, therefore, to speak of trypsin-inhibitory power or substance, more especially as the affix "anti" in "antitrypsin" is connected with the sense of being an antibody.

Lastly it is obvious that the explanation of the protection of organs against the action of ferments cannot be found by investigating the so-called "anti-tryptic titre."

## SUMMARY

1. The implantation of a piece of omentum into the intestinal lumen, thereby presenting a ready surface of absorption to the contents of the bowel, e.g., trypsin, did not cause any change in the "antitryptic" titre.

2. Thyro-parathyroidectomy, methyl-guanidine injections and administration of croton oil, all having a possible influence on the permeability of the intestine, have no effect on the "antitryptic" titre in normal dogs and those with omental implants.

3. Injection of commercial trypsin solutions or pancreatic juice, either subcutaneously or intraperitoneally, produces no change in the "antitryptic" titre. Nor does inoculation of whole pancreatic tissue produce any change. The presence of increased amounts of other ferments (e.g., leucoprotease, cell ferments, etc.) in the body as it occurs in septic and aseptic diseases, is equally without influence.

4. The sera of chicken, duck, goose, and pigeon possess a positive "antitryptic" titre nearly equal to that of dogs. Earthworms on extraction in saline, were also found to have marked "antitryptic" properties.

5. It was concluded, therefore, that trypsin does not act as an antigen and that the trypsin inhibiting factor of the serum is not an antibody. This confirms the work of Young, Opie, Jobling and others.

6. It is shown that the titre is not dependent on the viscosity of the sera, nor upon changes in the surface tension produced by saponin.

7. It is suggested that the "antitryptic" titre is the product of two components, one a proteolytic ferment (protease) and the other a tryptic inhibitory substance.

8. The survival of living tissue (omental implants) exposed to the action of trypsin, does not depend upon immunization against the ferment, but on some unknown factor, which may be local.

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# INTRAGASTRIC LEUCOPEDESIS

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The presence of leucocytes in the gastric juice has long been noted but received no attention except in diseased conditions of the stomach, such as cancer, gastric ulcer or gastritis.

Within recent years an extensive study of the subject has been made by Loeper and Marchal, who describe the following.

(1923a). In the normal individual, the gastric juice contains about 1500 to 1800 leucocytes per cmm., during the first hour, and 2000 to 2500 per cmm. during the second hour after the ingestion of "125 grams" of a 1 per cent peptone solution. The cells are rapidly destroyed and are said to liberate a proteolytic ferment which enhances the action of the pepsin in the juice.

(1922a). After filtration the juice exhibits a more feeble proteolytic action than before, the difference is believed to be due to the removal of of the leucocytes.

(1922b). The cells are chiefly polymorphonuclear except where there is hypochlorhydria, when lymphocytes are mostly found.

(1922c). Marked leucopedesis occurs after the ingestion of starches. The leucocytes then act as phagocytes of the starch granules and also assist the hydrolysis of the starch.

(1922d, 1923b, c). Glucose, saccharose, calcium chloride, sodium chloride, vinegar, aperitives, tea and bitters (e.g., quassia, calumba, gentian, nux vomica) all induce gastric leucopedesis. Isotonic solutions provoke the leucocytic movement into the juice, while hypotonic solutions inhibit.

(1923d). The leucocytes are not reabsorbed, but become destroyed and thus liberate their ferment.

(1924a). Eserine (parasympathetic stimulant) inhibits leucopedesis but excites (acid) secretion. Atropine has the contrary effect. The explanation of these actions is supposed to lie in the fact that the vagus stimulates secretion but inhibits leucopedesis.

It was also noted that after double vagotomy in the dog the leucopedesis to peptone solution increases five to six times (1924b). The sympathetics are supposed to inhibit secretion and increase leucopedesis.

Regarding the vagus influence on leucocytosis of the blood, Müller

(1922) has found the vagus dilates the splanchnic vessels and causes leucopenia in the peripheral blood, while the sympathetic constricts the splanchnic vessels and causes leucocytosis at the periphery. This is confirmed by Filinski (1924) who found that stimulation of the vagus causes an increase in the white count of the mesenteric blood while stimulation of the sympathetic results in a diminution. Glaser (1923) believes that in hemoclastic crisis the leucocytosis depends on a vagus reflex. Ciaccio (1922, 1923), Marino (1923), Fernandes (1922), Pagniez and Plichet (1923), Mirabile (1924), Bath (1924) and Cipriani (1924) found that digestive leucocytosis follows the ingestion of HCl. Cipriani even believes that the leucocyte count is directly proportional to the quantity of hydrochloric acid secreted, and Fernandes states that the ingestion of sodium bicarbonate produces leucopenia but this is denied by Bath.

On the other hand, the work of Kobryner (1924), who made 722 single observations during a total of 370 hours, shows that neither digestion nor food assimilation has any influence on the "leucocyte curve," which is normally periodic.

Pavlovsky (1920) has observed in a dog with a gastric fistula, that the injection of an emulsion of pancreas leads to a lymphocytosis and an increase of over 50 per cent in the pepsin content of the gastric juice, while injection of spinal cord emulsion produces a polynuclear leucocytosis but no effect on the proteolytic activity of the gastric juice. Loeper and Derbay (1922) reported that after meals, the pepsin content of the blood is increased, and Loeper and Baumann (1922) further state that the concentration of "pepsin" in the blood is roughly parallel to that in the gastric juice. Recently Ohno (1924) has found that an emulsion of lymphocytes freshly obtained from the mesenteric glands or the solitary lymph nodes of the intestine has a marked activating power on the proteolytic activity of the pancreatic juice, and he believes that they may also activate the enzymes of the gastric and the salivary juices.

The object of the present work was to determine the relation between gastric leucopedesis and the secretion of gastric juice and the relation between the blood leucocytes and those in the gastric secretion, especially with regard to the hypothetical rôle of leucocytes in the production and secretion of pepsin.

Methods. Heidenhain-, Pavlov- and splenectomized Pavlov-pouch dogs were employed, the stomach being made to secrete either by histamine (0.2 mgm. per kilo subcutaneously) or by meal (200 to 300 grams raw beef and 100 cc. water).

Juice cell counts were made with the Neubauer blood counting chamber, the juice being examined as soon as it issued from the fistula. Smears, fixed with formaldehyde vapour, and then stained with Wright's were used for the differential counts.

Blood counts were made from blood obtained by heart puncture, with one exception, when blood from the saphenous vein was taken.

Pepsin estimations were carried out with both the Hata and Mett-Schultz methods. With the former, the amount of substrate and juice used was constant, while acid was added to make a constant value (pH determinations being made after every test), the time of digestion being the variable employed for estimation. Each lot of egg-white suspension was standardized against Merck's pepsin and the results of the estimations calculated in terms of milligrams, Merck's pepsin according to the formula, $P = \frac{K}{t}$, $K$ being ascertained for each lot of substrate. With the Mett-Schultz method the results are given in millimeters[2]. Hata's method was employed as it is a more sensitive indicator of small amounts of pepsin than Mett's, but the latter is the more accurate for higher concentrations of pepsin.

The free and combined acids were estimated in the usual manner by titration against dimethylaminoazobenzene and phenolphthalein. Both the percentage and the output (i.e., the amount of HCl secreted per hour) are given.

RESULTS. *Leucocytes in the basal secretion.* It is found that normally under basal or interdigestive conditions there is a constant influx of leucocytes into the lumen of stomach (of all dogs irrespective as to whether the nerve connections are normal or not, i.e., whether the gastric pouch is a Pavlov or a Heidenhain one). The number varies from 35 to 502 cells per cmm. according to whether the juice is watery or mucous, i.e., according to whether there is much or little acid secretion (fig. 1), the average of 50 observations being 250 cells per cmm. The cells consist of 70 to 95 (average 87) per cent polymorphonuclear leucocytes, 3 to 22 (average 9) per cent mononuclear, and 0–8 (average 4) per cent epithelial cells. They can readily be seen in a fresh smear, made from the juice as soon as it drops out of the fistula. The cells thus obtained show no movements and are probably dead, being killed by the HCl. Their shape and their nuclei are well enough preserved, but stain imperfectly.

*Leucocytes in the active (histamine and meal) secretion.* In all eight histamine experiments there was invariably a sharp fall in the concentration of the cells from the control values, the intensity of the fall varying in-

Fig. 1. The effect of histamine. The total output of juice, HCl, pepsin and cells in the gastric secretion per hour is represented in columns, and the concentration in curves. The upper continuous line indicates pepsin, while the broken line represents the cell count per cubic millimeter. The lower continuous curve represents total acidity. The arrow indicates the time of injection. The lettering above each section denotes the animal number ($P$ = Pavlov pouch, $H$ = Heidenhain pouch, $S$ = splenectomised) and the experimental date.

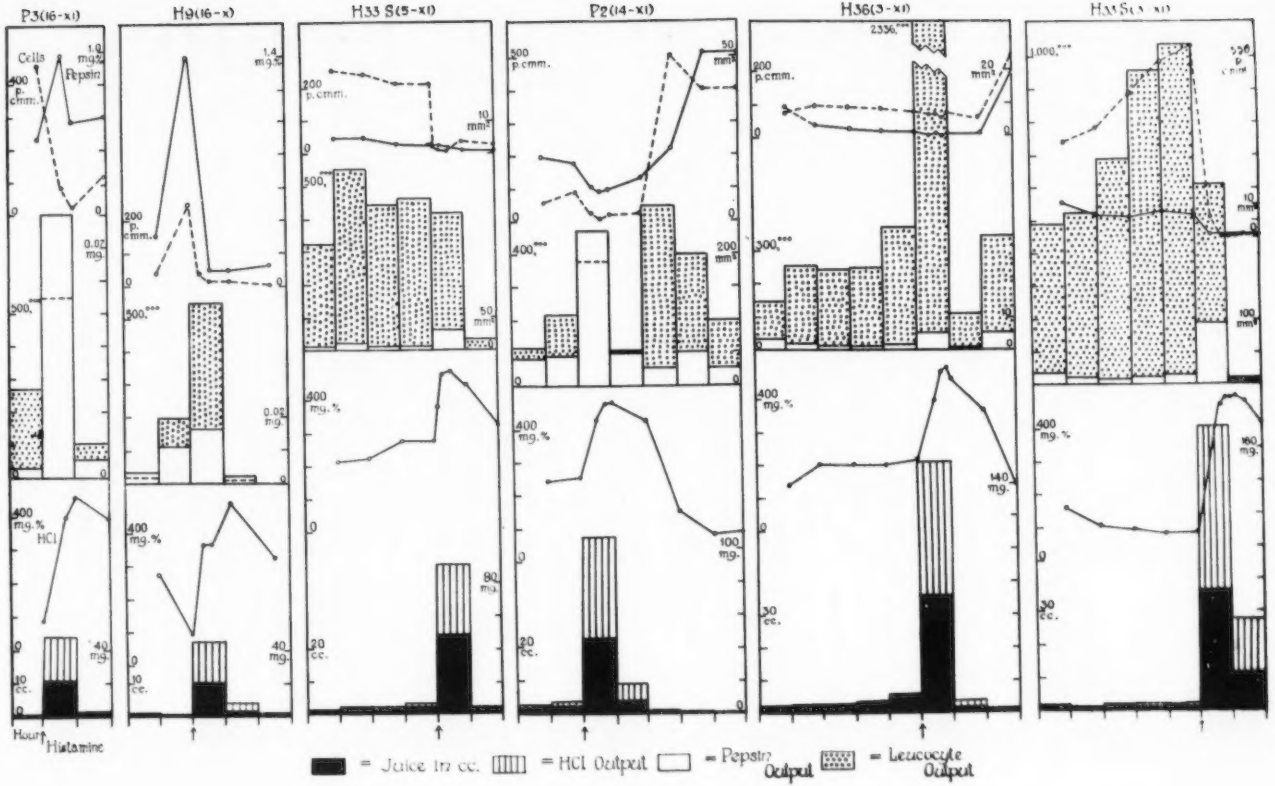
P3(16-xi)
H9(16-x)
H33 S(5-xi)
P2(14-xi)
H36(3-xi)
Cells
Pepsin
HCl
Hours
Histamine
= Juice in cc.
= HCl Output
= Pepsin Output
= Leucocyte Output

Fig. 1

versely with the initial concentration. It commences within ten minutes after the injection and reaches a minimum within one-half to one hour. This minimum is in the neighborhood of zero. Thereafter, there is a gradual rise in the concentration of the cells but in about one-third of the observations, the rise is only slight even after the third or fourth hour (see fig. 1).

As to the total output of cells, however, there is in the majority of the experiments a true increase in the number of cells during the first hour of active secretion. In a small number of experiments, the total cell output fell during the first hour. During the second and third hours, the total number of cells was invariably below the control values.

In five experiments, the effect of feeding on the gastric leucopedesis was found to be in general the same as that of histamine injection with regard to the concentration of the cells in the juice. But here the fall is more gradual and does not reach such a low level. With the exception of one experiment (P-2), where the minimum almost reaches the base line, the minimum is from 45 to 157 cells per cmm. Consequently the total cell output (except in P-2) never falls below "normal." The rise occurs after the first or second hour and returns to normal by the third or fourth hour, or even later (see fig. 2).

These results are contrary to those of Loeper and Marchal, but as they apparently carried out no control observations on the basal juice, it may be that what they regard as an increase, is in reality the return to "normal" concentration following the depression.

As a matter of fact, they state that "la courbe de la réaction présente toujours une dépression à la première demiheure et *se relève* ensuite comme si la leucocytose initiale, banale, dépendait du contact, et la leucocytose secondaire, vraiment réactionnelle, de la nature produit ingéré." It must be pointed out, however, that Loeper and Marchal made all their observations on the entire stomach, while our experiments were made on the gastric pouch.

Neither section of the vagi nor removal of the spleen has any influence on intragastric leucopedesis (cf. Heidenhain-Pavlov-pouch dogs in fig. 1). This finding does not agree with Loeper and Marchal's supposition that the vagus inhibits intragastric leucopedesis.

As to the differential count of the cells of the gastric juice it is found that during active (either histamine or meal) secretion there is a slight increase of polymorphonuclear leucocytes (average increase over basal in 50 observations is 7 per cent) with a corresponding decrease of the mononuclear or epithelial cells. The increase is more marked in those cases where the initial count is low.

*The relation of leucopedesis to HCl secretion.* The fall in the cell count after histamine or feeding may be due to one or more factors. That it is

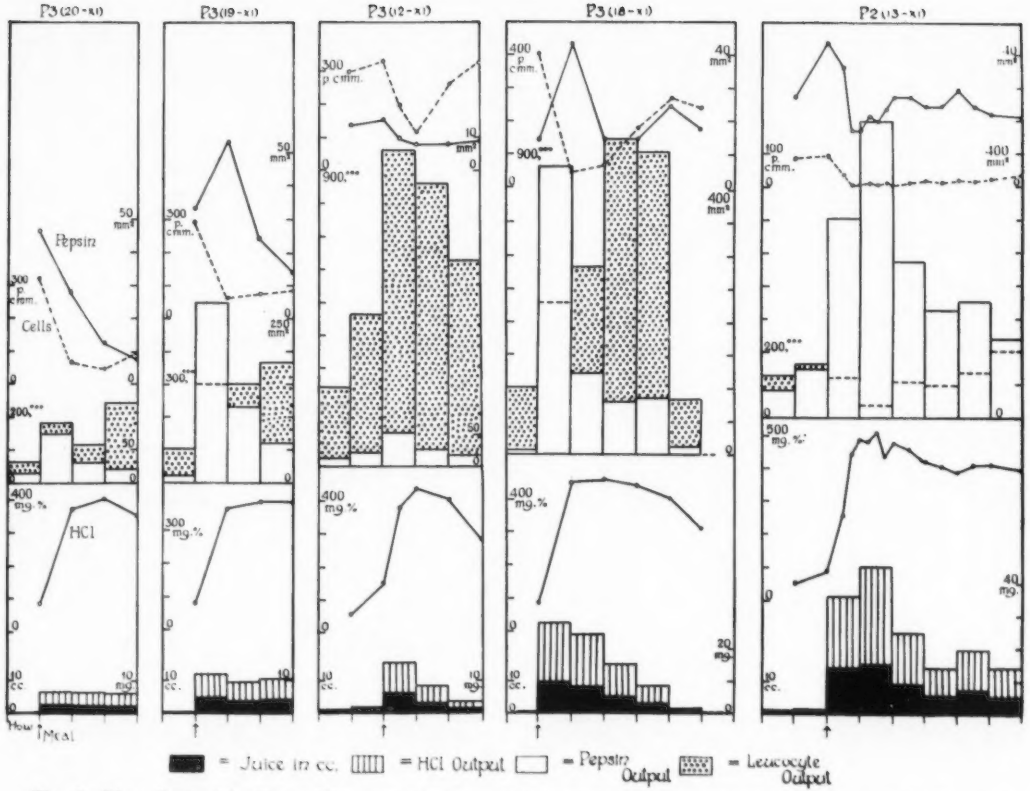


Fig. 2. The effect of feeding. The arrow indicates the time of feeding. Other particulars as in figure 1.

at first only a relative diminution is clear from the fact that the total output is increased, although later (during the second hour) the fall in percentage may be real in those cases in which the total output of cells is greatly diminished. It is obvious that the concentration of the cells is lessened by an increase in volume of the secretion. A comparison with either the HCl concentration or the output (figs. 3 and 4) shows that this is the most important factor, the curves of cell concentration being roughly inversely proportional to the curves of acidity.

*The relation of leucopedesis to pepsin secretion.* In most of the experiments there was a close parallelism between the concentration of cells and the concentration of pepsin in the juice, although the cell variations were always more extensive. As pointed out above, the fall in the cell concentrations is due to dilution by acid secretion; this is also the probable cause of the fall in pepsin concentration, as was observed by Lim and Liu (1926). The "total" pepsin content, however, is not identical with the total output of cells per unit time (hour), (see for example figs. 1 and 2). Thus out of thirteen observations, five show a rise of the cell count of from three to even ten times the control, and yet there was hardly any increase in the pepsin output. Another five observations show an increase of the pepsin output of from six to twenty times the control while the cell output did not in any instance increase to double the control. Only three observations showed a similar rise and fall in the cell and pepsin output per hour. From these findings we are unable to agree to the conclusion of Loeper and Marchal that the leucocytes are largely responsible for the proteolytic activity of the gastric juice.

To add further support to this objection, Loeper and Marchal's experiments with filtered and unfiltered gastric juice were repeated. Part of a freshly collected juice was filtered and the peptic activity was compared with the unfiltered portion, both being adjusted to the same volume and acidity and incubated at the same time. The following results were obtained:

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Filtered juice..... | 5.3 | 11.6 | 7.3 | 0.02 | 0.004 | 0.023 | 0.024 | 0.09 |
| Unfiltered juice..... | 5.8 | 10.9 | 6.8 | 0.02 | 0.004 | 0.023 | 0.026 | 0.13 |

Thus no loss of proteolytic activity after filtration was evident. (The first three results were obtained with the Mett method, the others with the Hata method.) It is possible, however, that the enzyme of the leucocytes may be set free immediately after the cell enters the gastric cavity (juice), in which event filtration would have no influence on the proteolytic power. To test this point the following experiment was made.

Figs. 3 and 4. Graphs from all experiments plotted together to show the relation between cells, acid and pepsin of the gastric juice, also the relation between cells and pepsin of the juice and the leucocytes and pepsin of the blood.

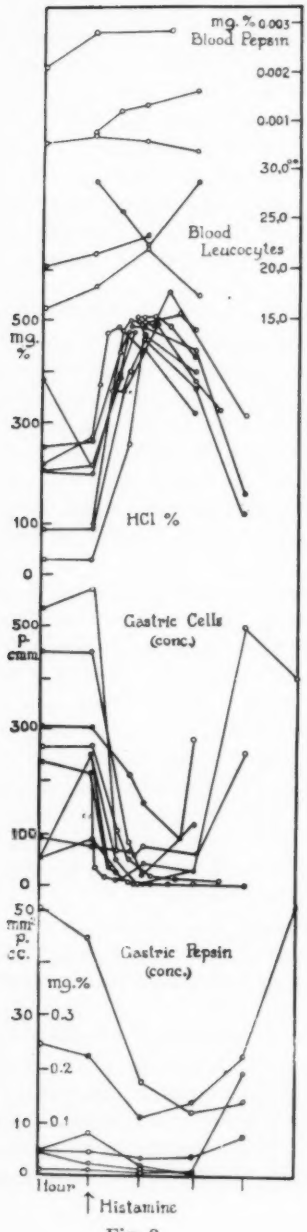
mg. %
0.003
Blood Pepsin
0.002
0.001
30,0
25,0
Blood
Leucocytes
20,0
15,0
500
mg.
%
300
100
0
HCl %
500
p.
cmm
Gastric Cells
(conc.)
300
100
0
50
mm²
p.
cc.
Gastric Pepsin
(conc.)
mg.%
30
0.3
0.2
10
0.1
0
Hour
Histamine

Fig. 3

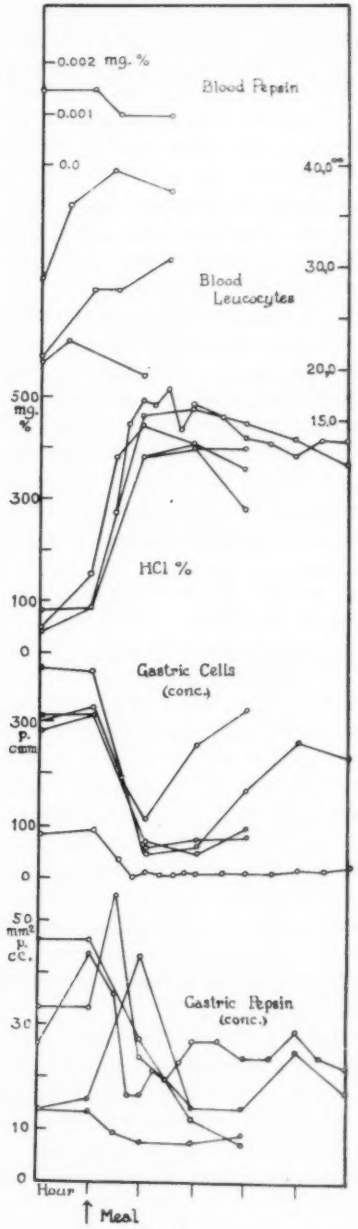
0.002 mg. %
Blood Pepsin
0.001
0.0
40,0
30,0
Blood
Leucocytes
20,0
15,0
500
mg.
%
300
HCl %
100
0
Gastric Cells
(conc.)
300
p.
cmm
100
0
50
mm²
p.
cc.
30
Gastric Pepsin
(conc.)
10
0
Hour
Meal

Fig. 4

A suspension of leucocytes (polymorphs 85 per cent, mononuclears 15 per cent) was prepared from the pleural exudate of a rabbit which had received an inoculation of an aleuronat broth mixture. After repeated washing in saline, a series of dilutions was made with distilled water, so as to reduce the amount of salt to the smallest possible quantity. These suspensions were then quickly added to different strengths of Merck's pepsin solutions, and the whole adjusted to an acidity of 0.2 per cent HCl, and made up to the same total volume (3 cc.) as had been employed for all pepsin determinations. The amount of salt in the leucocyte suspension was so small that any inhibitory influence on the digestion of egg white may be neglected.

The standard pepsin concentrations used in this experiment were 0, 0.1 per cent, 0.2 per cent, 0.3 per cent and 0.4 per cent, and the leucocytes added numbered 0; 100,000; 1,000,000; 2,000,000; 10,000,000; and 50,000,000 for each concentration of pepsin. Triplicate determinations were made in each series. It was found that without pepsin the leucocyte preparations exhibited no proteolytic activity while addition of various amounts of leucocytes gave no increase in the peptic activity. With larger quantities of leucocytes than 1,000,000 there was a slight decrease in the proteolytic activity probably in virtue of their action as foreign protein.

*The relation of leucopedesis to the leucocyte and proteolytic ferment content of the blood.* After meals, the usual (25 to 30 per cent) leucocytosis was observed in both the heart's blood and in the blood from the periphery. The increase commences within the first half-hour of feeding and continues for about three hours. This increase, however, does not coincide closely with the increase in the number of gastric leucocytes (fig. 4). From this, together with the results obtained after histamine when there is a very slight fall or no change at all, it might be concluded that there is no relation between the blood count and the migration of leucocytes into the gastric juice. That the increase in the blood leucocyte count during digestion cannot be due to the increase of HCl in the gastric juice is obvious from figures 3 and 4.

Both feeding and histamine have apparently no influence on the proteolytic ferment content of the blood, but since our method (Hata determination of the plasma) is possibly inadequate, no conlcusion can be reached at present.

It is clear, however, that there is no considerable amount of proteolytic ferment in the blood, and that the ferment contained in polymorphonuclear leucocytes is insufficient without further change or elaboration, to account for any variations in the proteolytic activity of the gastric juice.

Hence, it is difficult on the evidence available to accept any hypothesis which supposes that the polymorphonculear leucocytes are directly con-

cerned with the production of pepsin. Whether lymphocytes play any such rôle has not been determined, although in all the experiments some 10 per cent of the cells were mononuclear (lymphocytic?) yet no influence was observable.

## SUMMARY

1. There is a constant influx of cells, chiefly polymorphonuclear leucocytes, into the gastric lumen when the stomach is at rest (average 250 cells per cmm.). After histamine injections or meals the concentration of cells in the juice falls, reaching a minimum within 30 to 60 minutes, and then slowly recovers to normal in the course of two or three hours.

2. After histamine the total output of cells is raised during the first hour but lowered (below control values) during the second hour, while after meals the increase may be maintained for two to five hours, the subsequent fall not passing below the control, except in one experiment.

3. The fall in cell concentration is due mainly to the increase of HCl secretion.

4. Intragastric leucopedesis bears no constant relationship either to the peptic activity of the juice, or (apparently) to the proteolytic ferment content of the blood. There does not appear to be any connection between blood leucocytosis and leucopedesis.

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# STUDIES IN INCUBATION

## I. The Effect of Low Temperatures Previous to Incubation on Hatchability of Eggs Set

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*Contribution No. 5 from the Division of Poultry Husbandry, University of California Agricultural Experiment Station*

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On a number of occasions in recent years poultry breeders and hatcherymen in California have reported a marked decrease in the hatchability of eggs set following a cold wave in the late winter and early spring. The weather bureau records have shown that the temperature during these cold waves has rarely dropped lower than 25°F. at night and would then rise fairly rapidly after sunrise, reaching temperatures of 50°F. and higher in the early afternoon.

The eggs being saved for hatching are usually kept in unheated cellars or outbuildings, but due to the protection afforded by these buildings and the containers, such as egg cases and egg cabinets, in which they are packed, the eggs are not exposed to temperatures as low as those reached out of doors. It is very doubtful, therefore, if the eggs which have been reported by many breeders and hatcheries as hatching poorly following a brief period of very cold weather for the particular area concerned were ever subjected to temperatures below freezing or to such temperatures for more than a few hours out of each 24-hour period.

Colasanti (1) made studies of the effects of low temperatures on hatchability in 1875 and found that hen eggs that were immersed for one to two hours in an ice bath ranging in temperature from −4° to −10°C. during the time the eggs were in it and were practically frozen through when removed from the bath, showed apparently normal embryonic development after eight days of incubation as compared with control lots of eggs which had not been immersed in the ice bath.

Rabaud (2) found that subjecting fertile hen eggs to temperatures extending as low as −15°C. by immersing them in a mixture of ice and salt for one-half hour until they were practically frozen through (as indicated by the fact that a number of the shells cracked) did not prevent embryonic development when the eggs were removed from the ice bath and incubated for three days. The living embryos, however, were found to be abnormal. There was apparently a proliferation of cells without

distinct differentiation. Whether the eggs were thawed rapidly or slowly after being removed from the ice and salt mixture, did not seem to have any effect on embyronic development.

Mancini (3) obtained results somewhat similar to those obtained by Colasanti. He found that the exposure of hen eggs to a temperature range varying between $-4°$ and $-10°$C. for a period of two hours by immersing them in a refrigerating mixture transformed the contents of the eggs into a solid mass but did not injure the vitality of the germs and their power to develop. He concluded that the germ, while dormant, is less sensitive than after development has begun.

Lewis and Thompson (4) found that hatching eggs produced after a blizzard were low in fertility and embryos of the fertile eggs subnormal.

Elford (5) exposed a number of lots of hatching eggs packed in different ways to temperatures ranging from 14° to 26°F. for fifteen minutes to five hours and obtained results indicating that strong germed eggs will stand more cold than it has been considered safe to expose them to.

Mauro (6) found that eggs kept in a refrigerator at 32.9°F. for 24 hours were not appreciably affected. But when held at this temperature for 48 hours, the capacity of the embryos to develop was considerably reduced and after 72 hours of such refrigeration, it was entirely destroyed.

Mussehl and Bancroft (7) obtained data from their experiments indicating that exposure of hatching eggs to a temperature of 32°F. for 6 to 18 hours did not lower their hatching power or result in an unusual number of crippled or otherwise abnormal chicks. There was also found no observable difference in the vitality of the chicks that were hatched. When hatching eggs were held at a temperature of 32°F. for as long as 36 hours, however, their data do indicate a reduction in hatching power for the one lot chilled for a period of this length.

EXPERIMENTAL. The hatching eggs used in this experiment were held in an egg cabinet in a well-designed basement egg room where the temperature remained between 50° and 60°F., and were turned daily. None of the eggs were more than 3 days old. In preparing them for the experiment they were divided into 3 lots of 50 eggs each and 2 lots of 150 eggs each in such a way that each lot contained a proportionate number of each hen's eggs used.

In order to simulate, as far as possible, the conditions to be met with in the field, the eggs used in this work were not put into the refrigerator until 5 p.m. each day and were removed at 7 a.m. to an adjacent room having a continuous temperature of approximately 60°C. The refrigerator temperature ranged between 28° and 32°F.

In the valley and coastal areas which embrace the major part of the poultry industry of California, there is usually a marked spread in temperature over each 24-hour period. It may become quite cold at night,

although the late winter and spring temperatures rarely fall below freezing, and then with the rising of the sun the thermometer begins to mount fairly rapidly and may register from 50° to 85°F. at 2 p.m. Hatching eggs on commercial poultry farms would, therefore, be subjected to temperatures approaching the freezing point only during the very early hours of the morning.

Lot 1 was put in the refrigerator for 1 night, lot 2 for 2 nights and lot 3 for 3 nights. Lot 4 was held at a constant temperature of 40°F. and lot 5 at a constant temperature of 50° for 6 days or 144 hours. Lot 5 served as the control.

As indicated in table 1, the eggs placed in the refrigerator for 1, 2 and 3 nights respectively hatched better than those held at higher temperatures.

TABLE 1

*Results of two trials*

| LOT NUMBER | NUMBER OF EGGS | TIME EXPOSED | TEMPERATURE EXPOSED TO | PER CENT INFERTILE | PER CENT DEAD GERMS | | PER CENT DEAD IN SHELL TO FERTILE EGGS | PER CENT HATCHED TO FERTILE EGGS |
|---|---|---|---|---|---|---|---|---|
| | | | | | First test | Second test | | |
| First trial | | | | | | | | |
| 1 | 50 | 1 night | 28–32°F. | 8 | 10.87 | 6.52 | 26.08 | 56.52 |
| 2 | 50 | 2 nights | 28–32°F. | 12 | 13.63 | 9.99 | 20.45 | 56.82 |
| 3 | 50 | 3 nights | 28–32°F. | 18 | 4.88 | 12.19 | 17.07 | 65.85 |
| 4 | 150 | 6 days | 40°F. | 14 | 17.07 | 9.30 | 20.15 | 53.56 |
| 5 | 150 | 6 days | 50°F. | 12 | 12.89 | 10.6 | 22.72 | 53.79 |
| Second trial | | | | | | | | |
| 1 | 50 | 1 night | 28–32°F. | 18 | 14.63 | 7.31 | 14.63 | 63.41 |
| 2 | 50 | 2 nights | 28–32°F. | 6 | 12.76 | 17.02 | 19.14 | 51.06 |
| 3 | 50 | 3 nights | 28–32°F. | 16 | 14.28 | 7.14 | 23.81 | 54.76 |
| 6 | 100 | 3 nights + 38 hours | 28–32°F. | 6 | 10.63 | 11.69 | 21.27 | 56.38 |
| 7 | 50 | 4 nights + 38 hours | 28–32°F. | 6 | 6.40 | 10.63 | 34.04 | 48.97 |
| 4 | 150 | 6 days | 40°F. | 13.33 | 14.61 | 8.46 | 20.00 | 56.92 |
| 5 | 150 | 6 days | 50°F. | 6.66 | 10.71 | 5.71 | 16.43 | 67.14 |

The lots held in the refrigerator for 1 and 2 nights gave similar results in the per cent of chicks hatched to fertile eggs and these results were approximately 3 per cent higher than was obtained from the 2 lots held at 40° and 50°F. respectively, while lot 3 which was in the refrigerator for 3 nights hatched considerably better than any of the other lots.

In the second trial, two additional lots were used. One of these lots (lot 6) was put in the refrigerator for 4 successive nightly periods (being removed at 7 a.m. to a room temperature of 60°C. and returned to the refrigerator at 5 p.m.) and then, instead of being removed at the end of

the 4th nightly period, was left in the refrigerator for an additional continuous period of 24 hours. The other lot (lot 7) was put in the refrigerator for 5 successive nightly periods and then left in the refrigerator for an additional period of 24 hours as was done with lot 6. Lots 6 and 7 were therefore subjected to a temperature range of 28° to 32°F. for a continuous period of 38 hours immediately following their being exposed to such temperature conditions for 3 and 4 nightly periods respectively.

The hatching results of this second trial are given in the second part of table 1. These results together with the average results given in table 11 do not reveal any significant lowering of germinal vigor due to low temperatures for from 1 to 3 nights plus a continuous period of 38 hours. The lot held at 28° to 32°F. for 4 nights plus a continuous period of 38 hours, however, did show a drop in per cent of chicks hatched to fertile eggs.

TABLE 2

*Average results of both trials*

| LOT NUMBER | NUMBER OF EGGS | TIME EXPOSED | TEMPERATURE EXPOSED TO | PER CENT INFERTILE | PER CENT DEAD GERMS | | PER CENT DEAD IN SHELL TO FERTILE EGGS | PER CENT HATCHED TO FERTILE EGGS |
|---|---|---|---|---|---|---|---|---|
| | | | | | First test | Second test | | |
| 1 | 100 | 1 night | 28–32°F. | 13 | 12.75 | 6.91 | 20.35 | 59.96 |
| 2 | 100 | 2 nights | 28–32°F. | 9 | 13.19 | 13.50 | 19.79 | 53.94 |
| 3 | 100 | 3 nights | 28–32°F. | 17 | 9.58 | 9.66 | 20.44 | 60.30 |
| 6 | 100 | 3 nights + 38 hours | 28–32°F. | 6 | 10.63 | 11.69 | 21.27 | 56.38 |
| 7 | 50 | 4 nights + 38 hours | 28–32°F. | 6 | 6.40 | 10.63 | 34.04 | 48.97 |
| 4 | 300 | 6 days | 40°F. | 13.66 | 15.84 | 8.88 | 20.07 | 55.24 |
| 5 | 300 | 6 days | 50°F. | 9.33 | 11.80 | 8.15 | 19.57 | 60.46 |

Discussion. The claim that the hatching quality of eggs being held for incubation is seriously impaired by such brief periods of low temperature as may occur in the poultry districts of California during late winter and early spring is not substantiated by this investigation. The experimental lots of eggs were undoubtedly subjected to much more sudden and extreme changes in temperature in being put into and taken out of the refrigerator than would be the case on commercial poultry farms. They were also exposed to longer periods of low temperature. Yet the hatching results do not indicate that being subjected to such conditions seriously lowered their hatching quality as compared with the lots of eggs held at temperatures of 40°F. and 50°F.

The hatching data do, however, partially agree with the results obtained by Mauro and by Mussehl and Bancroft in that the lot of 50 eggs in the 2nd trial which was in the refrigerator for the longest period (4 nights plus a continuous period of 38 hours) did show a considerable reduction in hatching power as compared with the other lots.

Long-continued exposure to low temperatures approaching freezing probably is detrimental to eggs used for hatching but our data do not indicate that the periods of low temperature to which hatching eggs are likely to be exposed in the valley and coastal areas of California will lower their hatching qualities. As suggested by Mussehl and Bancroft, the effect of winter weather conditions on the breeding stock probably has more to do with any reduction in hatchability of the eggs laid than the temperatures the eggs themselves may be exposed to in this state.

## CONCLUSIONS

1. Exposure of hatching eggs to temperatures of 28° to 32°F. for 1 to 3 successive nightly periods of 14 hours each did not seem to have any detrimental effect on their hatching quality.

2. Exposure of hatching eggs to temperatures of 28° to 32° for 3 successive nightly periods plus a continuous period of 38 hours did not result in any significant reduction in the per cent of chicks hatched.

3. Exposure of hatching eggs to temperatures of 28° to 32° for 4 successive nightly periods plus a continuous period of 38 hours did result in a reduction in the per cent of chicks hatched.

4. A sufficiently long exposure to a temperature of 32°F. or to temperatures below this point will probably have a detrimental effect on the hatchability of hen's eggs but the brief periods of low temperature to which hatching eggs may be exposed in the poultry districts of California are evidently not cold enough or of sufficient duration to cause any serious injury to hatching eggs.

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# EARLY GROWTH RATES OF CHICKENS WITH SPECIAL REFERENCE TO ULTRA-VIOLET LIGHT

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Although it is now possible to rear chickens indoors without sunlight, many of the published "normal" growth rates (for which papers given in the literature list may be consulted) are submaximal. The best growth rates (fig. 4) are those published by Buckner, Wilkins and Kastle (4b) for 50 chicks brooded with hens, and those of Bethke and Kennard (3) who report a weight of 279 grams at 6 weeks and 596 grams at 9 weeks for a group of 21 chicks. Mussehl and Bancroft (19) report weights of approximately 300 grams at 6 weeks and 500 grams at 9 weeks for groups of 35 chicks, when the weaklings that appeared during the first week were eliminated. The Storrs (21) data are 268 and 526 respectively. Other "normals" are lower. Whether or not submaximal growth is to be regarded as subnormal may be open to discussion, but in as much as any machine operates at its best only when all conditions are optimal, it is reasonable to assume that when poultry growth rates are submaximal, environmental conditions are suboptimal. Parasites may be present, physical factors such as light, temperature, humidity may exceed the limits suited for best development, or the diet may be more or less insufficient or inadequate. It is possible then, that only a maximal growth rate is to be regarded as wholly normal. In rats Osborne and Mendel have recently reported an extraordinary acceleration over what was supposed to be normal growth rates.

Unpublished data gathered at Mount Hope Farm for several years past, on over four thousand chicks annually, show that when the growth of chicks is measured by weight at 30 days of age, those hatched in May grow less rapidly than those hatched in late March or early April (table 1). The same phenomenon was observed in Rhode Island Reds at the Massachusetts Agricultural College. Unpublished correlations show that in White Leghorns there is a marked correlation between maturity as measured by age in days when the first egg is laid, and hatching date. It has been shown by numerous workers that there is a definite correlation between maturity and egg yield. Consequently since the later hatched chicks grow more slowly they mature at an older average age, and so are

less productive. Atwood (1) has shown that when chicks are mistreated, they are less productive as adults under the best of care than birds which were given the best possible care from the egg on. Therefore, the growth rates of chicks become a factor of importance in studies on the physiology of egg production.

Space cannot be taken here to review the several hypotheses that have been considered in attempting to account for the observed variation in growth rates of chicks with season, nor has a solution of the problem been reached, but with the exception of those secured by Bethke and Kennard (3) or Buckner, Wilkins and Kastle (4b) early growth rates superior to those reported in the literature have been obtained. However, it is probable that we do not yet know how to secure the most favorable conditions for growing chicks.

In the course of this work, Mount Hope Farm undertook a number of experiments during 1925 and 1926, which while not bearing directly on maximal growth, cover points on which the literature is silent. This report deals mainly with growth rates as shown in these and other experiments at Mount Hope. Other studies made in coöperation with Dr. José F. Nonidez of the Cornell Medical College will deal with morphological features.

MATERIAL AND METHODS. Single-comb White Leghorns have been used in all the experiments. The chicks were not pedigreed but were the descendants of stock that has been pedigreed for several generations.

Because it is necessary to work as circumstances permit the present report is incomplete in several obvious respects which we hope to supply later, but in as much as they afford definite information on certain points on which the literature is silent, they are included here.

In general the experiments have followed two lines, one dietary, the other physical, the former being undertaken largely as a matter of orientation.

The experiments were conducted in a brooder house, 14 × 20½ feet, which was lighted by a large window on the north, and by three small windows and a glass door on the south. The intensity of the light from the south was diminished by an awning. During the second series, because of the high temperature outside, it was necessary to have the windows wide open, but care was taken to prevent direct sunlight from reaching the chicks. The same precautions were taken at other times when it was necessary to open windows for ventilation. That the diffuse radiation from the sky was without definite effect is attested by the behavior of the non-irradiated chicks (fig. 2) of the second experiment. A coal-burning brooder stove was placed near the center and four similar wire pens were provided, each with a corner projecting beneath the deflector. As Nelson and Steenbock (20) had reported that rats, themselves not irradiated,

confined in pens with rats that were being irradiated grew as well as the latter, the pens were so arranged that they were a foot apart. For the same reason, separate utensils were provided for each pen and care taken that nothing from one pen should reach another. After it was learned that contamination did not occur with poultry, these precautions were no longer observed.

The basic ration consisted of equal parts of wheat, oats and corn except in the first series when a commercial scratch feed, containing about 65 per cent of yellow cracked corn, the remainder being wheat, oats, millet, pease, Kaffir-corn and buckwheat was used, replaced when necessary by a commercial intermediate food containing about 50 per cent yellow corn. The dry mash in all experiments contained 50 pounds of bran, 20 of yellow

TABLE 1

*The influence of age of parents and of hatching date on weight of chicks at 30 days of age*

| HATCHED 1925 | AGE OF PARENTS | |
|---|---|---|
| | Two or more years | One year |
| | Weight of chicks | |
| | *grams* | *grams* |
| March 25 | 176 | 171 |
| April 1 | 179 | 163 |
| April 8 | 187 | 172 |
| April 15 | 180 | 159 |
| April 22 | 162 | 142 |
| April 29 | 166 | 141 |
| May 6 | 162 | 148 |
| May 13 | 135 | 124 |
| May 20 | 144 | 133 |
| May 27 | 123 | 112 |

cornmeal, 20 of meatscrap, 20 of sifted ground oats and 20 of white middlings. The meatscrap contained about 12½ per cent of bone which is equivalent to 2 per cent of the mash. The chicks ate at will of mash, with the scratch fed three to five times daily according to age. No green food was used.

The weight of each group was taken at the start and weekly thereafter to the close of the experiment. The food consumed was weighed in the first experiment but not thereafter.

Mention has been made of the influence of season on early growth of chicks. A second factor is the age of the parents as table 1 shows. These factors must be considered in interpreting the experiments.

Four series of experiments have been run thus far, as follows. (Table 2.)

Although 94 chicks were started in three lots in the first series, they were subdivided evenly at the end of three weeks because leg-weakness appeared,

the non-irradiated chicks into three groups, one being continued with the same treatment, the other two receiving sunlight, one in a box, the other on the ground. Two divisions were made of the cod liver oil lot, one receiving an addition of 5 per cent bone meal to the ration, the other receiving 5 per cent of $CaCO_3$ (ground limestone). Half the irradiated mash chicks were continued as started, the others received a milk supplement.

The machine used throughout these experiments for supplying artificial ultra-violet light was a Cooper-Hewitt mercury vapor lamp, designated as the "Uviac Laboratory Outfit" with rectifier. It was operated on 110 volts and 4 amperes and was run at least five minutes before exposures were made. The machine had been operated about one hour daily for about three months when the first series was started. At the beginning

TABLE 2

*Date of experiment, number of chicks and age of parents*

| SERIES | DATE BEGUN | NUMBER OF CHICKS STARTED IN EACH LOT | AGE OF PARENTS |
|---|---|---|---|
| | *1925* | | |
| 1 | February | 94* | Both old and young, but mostly under ten months of age |
| 2 | June | 50* | The same |
| 3 | October | 13 | Two and a half years or older |
| 4† | December | 14 | Six to eight months |

* But see below for exceptions.

† In the sixth and seventh weeks the chicks in this series suffered from a disease whose nature was not definitely ascertained, but appeared to involve an acute inflammation of the intestines. It subsided spontaneously after some mortality.

of the third series we were advised by the makers that it had probably lost about 25 per cent of its efficiency.

BASIC RATION. Growth on the basic ration is shown in figures 1 and 2. This ration has been supplemented either directly or indirectly in several ways, viz., milk, cod liver oil, or irradiated food, or the chicks themselves have been irradiated. The growth rates are shown in figures 1, 2 and 3. The milk was supplied ad libitum in the form of commercial condensed buttermilk (semi-solid). The cod liver oil was fed by thoroughly incorporating 2 per cent twice weekly in the dry mash which was before the chicks all the time. In the first series an unknown brand was used, in the second Patch's tested.

Two methods of radiating the food have been employed. In the first series, the dry mash was spread in a layer $\frac{1}{10}$ inch deep in zinc pans and stirred continually while exposed to the mercury vapor lamp at 2 feet for

10 minutes. A fresh supply was prepared twice weekly. In the second series exposure of both mash and scratch grain was made for half-hour to sunlight instead of the machine but otherwise the treatment was similar.

While a variety of exposures of the chicks themselves to the machine and to sunlight have been made, only those of the first two series are reported in this section for purposes of comparison with the basic ration. Studies of variation in length of exposure are reported in the later sections.

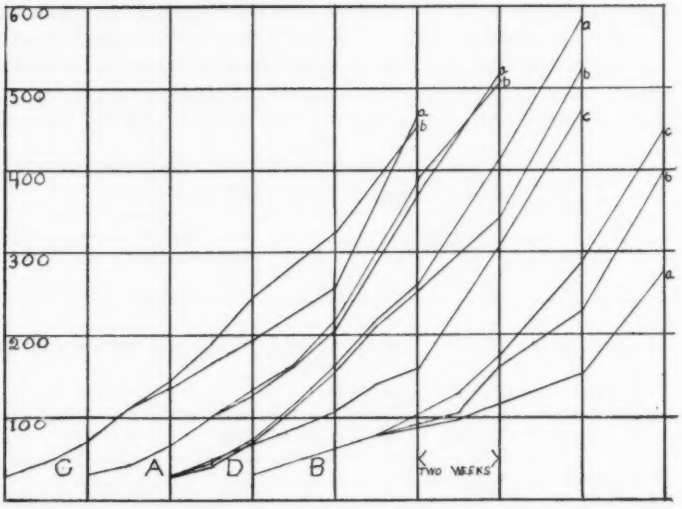


Fig. 1. First series, 94 chicks started in each group except D, where 31 chicks were started in each of three groups. At end of three weeks as explained in the text subdivisions were made. At the end of six weeks all chicks were transferred to a common house with outdoor run.

A. Cod liver oil. *Aa*, 5 per cent of bone meal. *Ab*, 5 per cent of limestone.

B. Basic ration alone. *Ba*, basic ration continued. *Bb*, sunlight with chicks in a box. *Bc*, sunlight with chicks on the ground.

C. Mash irradiated by machine. *Ca*, no change. *Cb*, buttermilk supplement.

*Da*, radiated 10 minutes daily with the machine. *Db*, radiated 20 minutes daily by the machine. *Dc*, Non-irradiated chicks in same pen with last two lots.

Two lots of 50 chicks each were sunned 30 minutes daily in a box with sides and top of wire, at 10:00 a.m., if possible, but exposure was given later if the sun was obscured at the set time. As far as possible exposure was continuous, but if the chicks showed signs of distress from the heat they were removed to the shade to cool off. One lot of 31 chicks was given 10 minutes' daily irradiation from the mercury vapor lamp while a second lot of 32 chicks received 20 minutes' daily irradiation. The growth rates

resulting from the use of the basic rations and from the several methods of supplementing it are shown in figures 1, 2 and 3. The semi-solid buttermilk was the most effective agent in promoting growth, being more effective than cod liver oil and about equal to half-hour irradiation by sunlight, but not quite so effective as irradiation by the machine. But when the butter milk was combined with cod liver oil or sunlight still better growth was obtained, better growth being obtained from cod liver oil than from

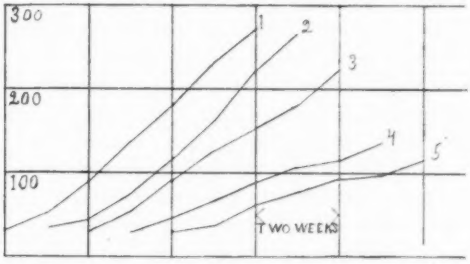


Fig. 2. Growth of non-irradiated chicks with and without milk.

*1*, fourth series, milk; 14 chicks. *2*, third series, milk; 13 chicks. *3* and *4*, second series, 3 milk, 50 chicks, 4, no milk, 50 chicks. *5*, first series, no milk, 94 chicks, reduced to 20 at end of third week.

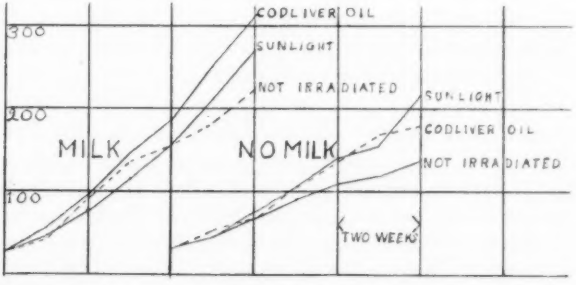


Fig. 3. The effect of a buttermilk supplement (semi-solid) on the growth of chicks

exposure to sunlight. Cod liver oil without milk gave no better growth than sunlight without milk. As 50 chicks were started in each lot differences due to random sampling are not likely to play a large part in bringing about the observed differences. Perhaps milk and cod liver oil supplement each other.

The chicks of the second series that received food exposed to sunlight for half an hour were benefited very little. At the end of 6 weeks there were 25 left alive with an average weight of 157 grams, compared with 22

survivors weighing 137 grams in the lot that received only the basic ration without irradiation, 218 grams for 25 survivors of the lot that received a buttermilk addition to the basic ration but which were not irradiated and 221 grams for the sunned chicks.

But when the mash alone was irradiated as was done in the first series better growth was obtained than without irradiation, but not as good as with irradiation of the chicks themselves (fig. 1). The difference between the effectiveness of sunlight as compared with the machine is in line with Hess, Weinstock and Sherman's observation that lettuce growing out of doors in May, June and July failed to protect rats against rickets, though exposure of the lettuce to the machine was effective.

As explained above, subdivisions were made at the end of 3 weeks, one subdivision being continued on the original program, the other receiving a butter milk supplement, with rather peculiar results, for at the end of 6 weeks the latter consisted of two distinct subgroups, one of strong, apparently normal chicks, the other of weak chicks whose condition approximated that of the non-irradiated group. The reason for the division is not clear, but as the mash was fed in hoppers, the buttermilk paste on a board placed vertically at the end of the pen away from the hover, and the scratch feed on the floor, the weaker individuals could satisfy their appetites most easily with the scratch feed and, being already predisposed to remain near the heat, may have failed to eat either mash or milk. The strong individuals were in very good condition, better than any in their comparison group and of about the same quality as those radiated by the machine, as is attested by the average weight at six weeks as shown in figure 1, *Cb*, which represents the combined weights of the strong and weak chicks.

*Contamination experiments.* Nelson and Steenbock (20) have shown that when rats, not themselves irradiated, have access to the excreta of irradiated rats, even in very small amounts, they develop normally. To test this point with chicks two experiments have been run. In the first series, 31 chicks were allowed to run in the same pen with the lots exposed for 10 and 20 minutes daily to the machine. At 6 weeks they weighed 157 grams compared to 261 for those irradiated 10 minutes daily by the machine. Access to the droppings of their irradiated mates was ineffective in promoting growth. Moreover, they developed leg weakness just as did those not irradiated at all.

In a second trial beginning on the fifth day of life, the droppings of irradiated chicks were air dried near the brooder, pulverized and thoroughly mixed with the dry mash supplied one lot of chicks. Although these chicks had a buttermilk supplement the effect of feeding the droppings was unfavorable rather than otherwise (fig. 5). Evidently chicks fail to excrete the anti-substance in their droppings in protective amounts.

*Effect of length of daily exposure to the machine.* The chicks used in these experiments were hatched October 2 from eggs laid by hens two and one-half years or older, a factor that must be borne in mind when considering the growth rates. They also received the buttermilk supplement to the basic ration.

All exposures were made daily at 3 feet from the tube of the machine. We were advised by the manufacturers that about 25 per cent of its original efficiency had been lost at the time the experiments were begun.

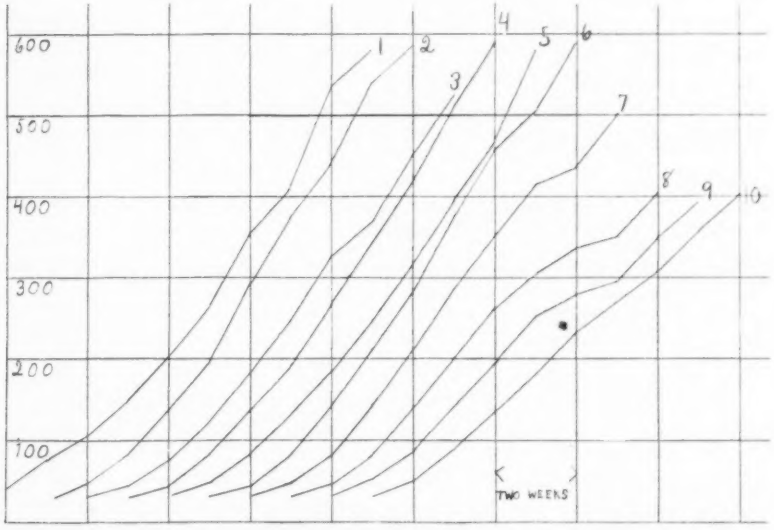


Fig. 4. Growth rates of chicks under varying lengths of daily exposure to the mercury vapor lamp. Buttermilk added to the basic ration. Third series, 13 chicks started in each group. The fraction attached to each group number records in the numerator the number of pullets alive at nine weeks, the denominator records the number of cockerels.

*1*, Buckner, Wilkins and Kastle's data. *2*, composite data for groups exposed 1, 2, 3, 6, 15, 30 and 60 minutes daily. *3*, (6/4) 120 minutes. *4*, (8/3) 10 minutes. *5*, (5/4) 10 minutes daily increments beginning with 10 minutes till 3 hours were given daily. *6*, (5/5) 1 minute. *7*, (6/5) 30 seconds. *8*, (5/5) 15 seconds. *9*, (4/4) 5 seconds. *10*, (4/5) not irradiated.

Exposures of the following length were made, 5, 15 and 30 seconds; 1, 2, 3, 6, 10 and 30 minutes; 1 and 2 hours. One lot was started at 10 minutes, and increased daily by this amount till a maximum of 3 hours at one exposure was made. Thirteen chicks were started in each group. The down was removed from the backs of two individuals in each group

and kept off as it was replaced by new growth, but beyond severe burns, which gradually healed, in those individuals exposed 30 minutes or longer nothing definite was noted.

The more important growth curves are shown in figure 4. The 5- and 15-second daily exposures give practically the same growth rates as no exposure. Thirty seconds daily is adequate or nearly so during the early weeks, but appears inadequate in the later part of the experiment though this is uncertain because of the small numbers involved. One minute per day is as adequate as the longer periods.

The seven groups exposed to the machine for 1, 2, 3, 6, 15, 30 and 60 minutes daily constitute a moderately large population (91 chicks) and as there is no indication that differences in growth rates due to length of exposure exist among the several groups, their averages (fig. 4) may safely be compared with others such as Buckner and his associates (4b) have pub-

TABLE 3

*Growth of chicks at several distances from and length of exposure to the mercury vapor lamp*

| DISTANCE FROM TUBE | LENGTH OF EXPOSURE | COCKERELS | PULLETS | WEIGHT AT 6 WEEKS | COCKERELS | PULLETS | WEIGHT AT 9 WEEKS |
|---|---|---|---|---|---|---|---|
| *inches* | *minutes* | | | *grams* | | | *grams* |
| | Not exposed | 7 | 5 | 276.7 | 5 | 4 | 437.7 |
| 3 | 10 | 3 | 10 | 226.9 | 3 | 8 | 407.1 |
| 16 | 10 | 6 | 7 | 266.9 | 6 | 6 | 433.4 |
| 24 | 10 | 6 | 8 | 273.6 | 5 | 8 | 460.1 |
| 36 | 10 | 5 | 7 | 286.3 | 4 | 5 | 446.5 |
| 50 | 10 | 7 | 7 | 311.4 | 5 | 7 | 447.1 |
| 70 | 10 | 6 | 8 | 290.0 | 4 | 7 | 453.0 |
| 3 | 1 | 4 | 10 | 270.3 | 3 | 7 | 497.0 |
| 70 | 1 | 4 | 7 | 285.9 | 3 | 5 | 471.5 |

lished (fig. 4) to which they are similar as are also those of Bethke and Kennard's 21 chicks (3). It may be mentioned that Buckner, Wilkins and Kastle included in their data only those chicks that grew to maturity, whereas our data include all chicks alive at each weighing. Our chicks did not continue growing at the rate indicated by their weight at 6 weeks, just why is not evident at present, but at any rate it appears possible to secure much greater growth rates than is often considered normal. It may prove to be a matter of significance that Bethke and Kennard's chicks as well as our own were hatched in October.

*Intensity of exposure.* The chicks were from young hens 6 to 8 months of age. In passing it may be remarked that a 70 per cent hatch was obtained, which is in contrast to Hart and associates, (11) surmise that in late fall a low hatching quality of eggs of 30 to 40 per cent is to be expected.

The intensity of the radiations received by the chicks should vary in-

versely with the square of their distance from the machine. It is also possible that the air may act as a differential filter.

Vertical distance of 3, 16, 24, 35, 50 and 70 inches from the tube were used with exposures of 10 minutes daily, and of 3 and 70 inches with one minute daily exposure.

To prevent the chicks that were 3 inches from the tube from being overcome by the heat, a current of air was circulated by means of an electric fan. Under these conditions they appeared comfortable. The results are condensed in table 3 and figure 5.

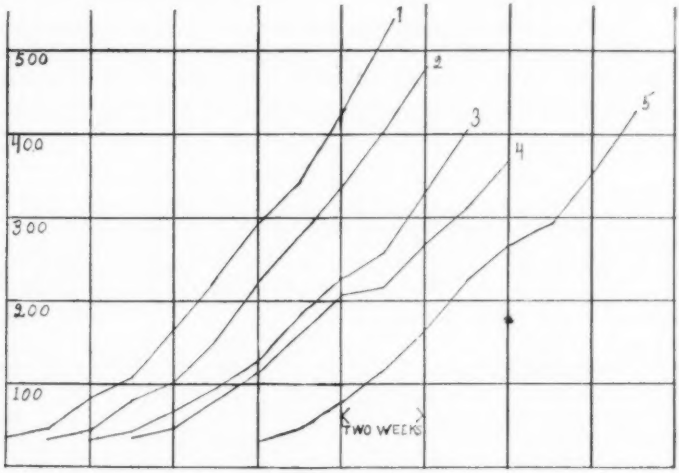


Fig. 5. Growth rates of chicks under various conditions. Fourth series. Buttermilk added to the basic ration, 14 chicks started in each lot. The fraction indicates pullets (numerator) cockerels (denominator) at 9 weeks.

*1*, (9/2) Winter sunlight at will. *2*, (6/2) One minute at 3 feet from the mercury vapor lamp twice weekly. *3*, (8/3) 10 minutes' daily exposure to the mercury vapor lamp at *3 inches* from the tube. *4*, (7/3) chicks not irradiated but droppings from radiated chicks added to the mash. *5*, (4/5) not irradiated.

The experiment seems to show that the distances from the tube employed are without marked influence on growth rate, except that there are some indications that too intense radiation over a sufficiently long period is unfavorable to maximum growth, for the 3 inch 10 minute lot was distinctly smaller than any of the others. The smaller size is not wholly due to the sex ratio, for the three inch one minute lot has a similar sex ratio, yet are among the largest chicks. It will be recalled that the lot exposed two hours daily grew less rapidly than their mates. Exposure to June sunlight for half an hour was also less satisfactory than expected. There is at least a hint of the existence of growth-inhibiting radiations.

*Frequency of irradiation.* Three experiments were run in the fourth series in which irradiations were given less often than once a day. The results are shown in table 4.

If the irradiation results either directly or indirectly in the formation of substances necessary for growth by the organism it appears that the quantity is insufficient when the chicks are radiated only once a week, but is adequate when radiated twice weekly.

*Dilutions of the radiations.* Several experiments were run in which substances were interposed between the source of the radiations and the chicks.

In the first the chicks' own down (or feathers as they grew older) was used. The object of the experiment was to learn whether sufficient radiation penetrated the loose coating of the chicks to promote normal growth. Ten chicks of the June lot were placed in an apparatus which permitted an exposure of their backs only. They received ten minutes daily doses at three feet from the machine. At 6 weeks the seven survivors weighed

TABLE 4

*The effect on growth of frequency of irradiation*

| LENGTH OF IRRADIATION | FREQUENCY OF IRRADIATION | DISTANCE FROM TUBE | COCKERELS | PULLETS | AVERAGE WEIGHT AT 6 WEEKS | COCKERELS | PULLETS | AVERAGE WEIGHT AT 9 WEEKS |
|---|---|---|---|---|---|---|---|---|
| *minutes* | | *feet* | | | *grams* | | | *grams* |
| None | | | 7 | 5 | 276.7 | 5 | 4 | 437.7 |
| 10 | Twice weekly | 3 | 7 | 7 | 341.0 | 5 | 6 | 491.6 |
| 10 | Once weekly | 3 | 6 | 8 | 279.4 | 4 | 5 | 361.5 |
| 1 | Twice weekly | 3 | 3 | 8 | 279.1 | 2 | 6 | 479.1 |

164 grams compared with 137 grams for the 22 survivors of the unexposed lots, and 261 grams for those irradiated in the usual manner.

In six experiments celoglass and white muslin were used. The former substance is a commercial product resembling celluloid, reinforced with wire screening having 14 wires to the inch. The muslin used had 70 threads to the inch.[1]

The results are summarized in table 5.

The experiments suggest that further tests may show cloths that transmit sufficient ultra-violet light and still are protective against unfavorable weather conditions.

[1] After this paper went to press it was learned through a lecture by Prof. L. F. Payne of the Kansas Agricultural College that his colleague, Prof. J. O. Hamilton of the Department of Physics had tested the transparency of celoglass and muslin to ultra-violet light and had found that "medium" white muslin transmits 33 per cent of ultra-violet light falling upon it and celoglass 25 per cent. It is of interest to note further that the fibres of the muslin are themselves partially transparent to ultra-violet light.

*The effectiveness of winter sunlight.* Hess (13) has expressed the opinion that winter sunlight in the latitude of New York City is insufficient to prevent rickets in children under general winter conditions. Whether this is due to absence of sufficient ultra-violet light, or to the reluctance of parents to expose their children to the sun sufficiently or to the smoke and dust prevailing around cities, or to winter climatic factors other than temperature is not clear. Winter sunlight might then be inadequate for young chicks. For this reason one lot of the December series was provided with means of access to sunlight. As it was not feasible to permit the chicks to run in and out the main building, a small box electrically heated was provided for warmth, and with free access to another box which had a large opening on the south covered with poultry wire, thus providing a place for exercise and feeding in the sun. At night the chicks were transferred inside with the others. There were 3 cockerels and 10 pullets which weighed

TABLE 5

*The effect on chick growth of diluting the irradiations*

| DATE | NUMBER OF CHICKS STARTED | SOURCE OF IRRADIATIONS | MATERIAL | LENGTH OF EXPOSURE | MILK SUPPLEMENT | NUMBER OF CHICKS | WEIGHT AT 6 WEEKS | NUMBER OF CHICKS | WEIGHT AT 9 WEEKS |
|---|---|---|---|---|---|---|---|---|---|
| | | | | *minutes* | | | *grams* | | *grams* |
| June | 10 | Sun | Celoglass | 30 | No | 3 | 146 | | |
| June | 10 | Sun | Muslin | 30 | No | 5 | 167 | | |
| June | 10 | Machine | Celoglass | 10 | No | 7 | 184 | | |
| June | 10 | Machine | Muslin | 10 | No | 7 | 195 | | |
| October | 13 | Machine | Celoglass | 30 | Yes | 11 | 396 | 10 | 689 |
| October | 13 | Machine | Muslin | 30 | Yes | 11 | 382 | 10 | 662 |

295 grams at 6 weeks of age and 536 grams at 9 weeks (fig. 5). They were better appearing than any of the others.

According to Dorno, as quoted by Hess (12), the sun's rays have no rays shorter than 308 millimicrons at this season of the year. As chicks develop leg weakness back of glass which transmits rays down to 312 millimicrons, it is apparent that if we may apply Dorno's data to Williamstown, chicks are capable of developing normally when receiving only the band of rays between 308 and 312 millimicrons.

*Bleaching of pigment by cod liver oil.* The cod liver oil lots in the second series showed an absence of yellow pigment in the beaks and shanks, which cannot be wholly due to lack of carotinoid pigment in the diet, since yellow corn was used and the normal yellow color was present in all the other groups. Dunn (6) (7), Bethke and Kennard (3) noted the same paleness in their birds reared in confinement on cod liver oil, but attributed it to

lack of carotinoids in the diet. At the close of the experiment proper, each of the two groups that received cod liver oil was subdivided into four parts, making eight in all; two groups (one from each of the two original groups) having yellow corn substituted for the mixed scratch grain, two receiving green food, two being allowed to run in the sunlight for several hours daily, the other two being held as checks. After two weeks, the oil was removed from the rations of one member (the one that had received no milk) of each pair. Pigment slowly developed in some individuals in each of the eight groups, but after five weeks some still remained pale. Hess (14) observed that ultra-violet light decolorizes a solution of carotin in the presence of cholesterol, suggesting that something in the oil tends to decolorize the yellow pigment in the birds fed cod liver oil.

*Milk.* When the present series of experiments were begun it was not known that milk under some circumstances may possess anti-rachitic properties, as has been recently shown by Gowen et al. (5) and by Steenbock et al. (21). Hart et al. (8) (9), had used skim milk in their experimental diets and found that while leg weakness was retarded, eventually the chicks succumbed which seemed to indicate that milk was lacking in the antifactor. But since milk may contain the anti-rachitic factor, its use renders the interpretation of the experiments of others as well as my own somewhat uncertain. The milk may contain small amounts of D, sufficient to tide the chicks along. But it is perfectly clear that the addition of D, either through radiation or in cod liver oil exerts a favorable influence over and above that of the milk in all except the fourth series. Here radiation was of doubtful benefit, possibly because the milk used in this series may have contained more D than that used in the other series. The favoring influence of the semi-solid buttermilk on growth is shown in figure 3. Each lot that received the milk grew faster than its companion lot. Milk was superior in promoting growth to either sunlight or cod liver oil for the period under consideration.

*Conclusions.* The experiments show that it is possible to obtain rates of growth in White Leghorn chicks considerably above those previously reported with the exception of those of Bethke and Kennard and Buckner, Wilkins and Kastle. From this it follows that earlier maturity may be expected, and since that is associated with greater egg production, the latter may be expected to show some increase.

It is also apparent that very small amounts of radiation are necessary for normal growth, which indicates that the use of cod liver oil as a source of vitamin D is wholly unnecessary in rearing poultry. It seems possible that further research will show that all the poultry man needs to do is to rearrange his brooding facilities so that sunlight can reach his chicks for a comparatively short period once or twice a week. Hart, Steenbock, Lepkovsky and Halpin (9) give tables which show that when using a ratio

deficient in A (i.e., a white corn ration) the same rate of growth was secured with 10 minutes' daily sunlight as with 10 minutes' radiation from the machine. As the latter is sufficient for normal growth, had they used yellow corn, the growth rate would doubtless have been normal on ten minutes' sunlight daily. In a preliminary trial with a few chicks on a normal diet, and no milk after the first 2 weeks, leg weakness did not develop in 5 weeks on 10 minutes' sunlight daily. When one considers that the small amount of naked skin about the bird's head is the main receptor of the radiations, since under the circumstances of the experiments with radiation from above, the legs are protected to a considerable degree, and the short time of exposure found necessary, it seems quite clear that some

TABLE 6

*Mortality at six weeks in several experiments*

| CONDITIONS OF EXPERIMENT | SERIES | PER CENT DEATHS |
|---|---|---|
| Cod liver oil | 1 | 9.5 |
| Cod liver oil | 2 | 6.0 |
| Cod liver oil and milk | 2 | 4.0 |
| Not radiated | 1 | 36.1 |
| Not radiated | 2 | 48.0 |
| Not radiated (milk supplement) | 2 | 42.0 |
| Machine radiated 10 and 20 minutes daily | 1 | 8.0 |
| Sun radiated | 2 | 34.0 |
| Sun radiated (milk supplement) | 2 | 32.0 |
| Mash machine radiated | 1 | 42.6 |
| Mash machine radiated (milk supplement) | 1 | 29.7 |
| Food sun radiated | 2 | 46.0 |

photo-chemical process must take place with great rapidity and that only minute amounts of the resulting active agent is necessary to maintain normal growth.

*Mortality.* The normal mortality of the stock is about 7 per cent in 3 weeks. That the reader may have the information, table 6 is given. It includes the mortality only in those groups where either the conditions of the experiment or the numbers involved warrant the belief that it is of significance.

What appeared to be rickets accounted for the deaths in those lots deprived of ultra-violet light, but it is difficult to explain the deaths in the second series which were exposed to sunlight. It might be due to vitamin A deficiency since the two lots receiving cod liver oil showed a low mortality. But no lesions suggestive of A deficiency were noted. The supply of yellow corn seems to have been adequate, as shown by the low mortality among machine irradiated chicks. Possibly the corn used in the second experiment had lost much of its A. But it is also possible that the chicks

may have been injured by too much heat, though they were removed to the shade if evidences of overheating appeared, as sometimes happened. The exposure box was well ventilated, nevertheless the sun in June at 10 a.m. is decidedly hot.

*Uniformity in growth.* Even when good average growth is obtained, it often happens that some of the chicks are much larger than the others. Some lots, even when fairly large, are quite uniform in size. Thus at five weeks of age the (50) chicks of the second series receiving cod liver oil and milk showed an average weight for the four largest of 312 grams and 255 for the four smallest, a difference of 57 grams, while in the lot of equal size receiving cod liver oil as the only supplement, the four largest were 255 and the four smallest were 106, a difference of 149 grams. Differences of the same order existed in all the other groups of this series. Evidently some chicks respond less favorably to conditions of the environment. Occasionally, exceptionally good growth is made by one or two individuals in a lot which as a whole react unfavorably to a special set of conditions. It would be very interesting to learn the physiological basis for such variation in individual response, for they may not be genetic, but result from some non-genetic factor, such as storage of certain vitamins in the egg, or some accidents of incubation.

*Leg-weakness.* Besides retarded growth, chicks deprived of ultra-violet light or its equivalent exhibit the condition called leg-weakness, supposed by some to be identical with mammalian rickets. In the first series some anomalous conditions were noted. Leg-weakness first appeared in the group receiving the basic rations at 12 days of age, and to our surprise within 3 days one or more individuals in each group were exhibiting this condition. Because of this, the experiments were modified by subdividing the groups as explained above continuing one subgroup on the line originally planned and modifying the other. The physical subdivison was accomplished by diagonal wire partitions.

Two types have been noted. In one, occurring mainly among the non-irradiated groups, there was no marked deformity of the legs. The chicks in the early stages exhibited a wobbly gait, and a little later seemed weak and able to move only with difficulty, progressing in a half-squatting manner as though unable to stand. They seemed content to remain resting on the floor. The second type occurred in the other groups. In its early stages it was no different, but soon the toes became curled toward the median plane, forcing the chick to walk on the outside of its feet. Besides the difficulty in locomotion due to the twisted toes, there seemed to be something of the same manifestation of weakness as in the other type but less intense. We were unprepared to encounter the two types of leg-weakness, though a figure published by Hart et al. (8) indicates that others have observed them. Possibly the second type has nothing to do directly with the lack of vitamin D but arises from some other deficiency. In the

cod liver oil lot, from the third to sixth weeks, a third of the chicks showed some leg-weakness, in the irradiated food lot there was about the same proportion, while in the machine radiated lots, about a fifth had some leg-weakness. Three-fourths of the non-irradiated chicks penned by themselves showed leg-weakness by six weeks, but only half of the non-irradiated chicks in the same pen with those that were irradiated exhibited this reaction. In the second series only 2 to 10 per cent of the chicks showed signs of leg-weakness at 6 weeks and they occurred in all lots. There was very little leg-weakness even in the lot that received nothing but the basic ration.

## SUMMARY

1. It is shown that early growth rates in poultry in excess of those often reported in the literature as "normal" may be obtained.

2. As measured by weight at 30 days of age, chicks hatched in the early part of the season grow faster than those hatched in the later part.

3. Chicks from hens two years of age and upwards are heavier at 30 days of age than those from hens one year of age.

4. Non-irradiated chicks penned with irradiated ones reacted as did non-irradiated chicks penned separately. Forced feeding non-irradiated chicks with the droppings of irradiated chicks as a further test of the presence of a growth promoting substance in the excreta of irradiated chicks was ineffective in promoting growth.

5. Irradiating the dry mash for ten minutes appeared to favor growth, but did not wholly prevent leg-weakness. Sunning the food for half an hour did not favor growth.

6. The addition of milk (semi-solid buttermilk) to the basic ration was very effective in promoting growth.

7. Daily exposures of five and fifteen seconds to the mercury vapor lamp were ineffective in promoting growth. Thirty seconds were not completely adequate but one minute gave as good results as exposures up to three hours.

8. There were some slight indications that prolonged, or very intense radiations from the mercury vapor lamp retarded growth.

9. Radiations for one and ten minutes, twice weekly, from the mercury vapor lamp at three feet gave normal growth. Less frequent radiations appeared to be inadequate.

10. The natural covering of the chicks is not penetrated by the effective wave lengths of ultra-violet light. Evidently the usual receptive areas of the rays are those parts that are normally exposed.

11. Effective ultra-violet light under some conditions penetrates muslin or celo-glass.

12. Winter sunlight appears to be both growth promoting and a preventive of leg-weakness.

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# THE REGULATION OF RESPIRATION

## V. A Continuous Thermo-Electric Method of Recording the Volume-Flow of Blood

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The significance of the blood as a carrier of the stimulus for the control of pulmonary ventilation was an accepted principle in theories of respiratory control. Witness the accumulation of data on the hydrogen ion concentration of the blood as related to pulmonary ventilation. The respiratory center according to theory responded to or was stimulated by the hydrogen ions of the circulating blood. That the respiratory center itself must have a source of energy for carrying on its own important activities seems to have been virtually overlooked. Consequently the study of the relation of the acid metabolism and resulting acidity of the respiratory center as affected by volume-flow of blood through the center gave way almost entirely to the study of the composition and behavior of the blood with respect to the hydrogen ion concentration of the blood itself.

Whatever the explanation may be the fact that hyperpnea of hemorrhage is markedly reduced by the reinjection of blood or the injection of a blood substitute indicated the need of another outlook on respiratory control. Particularly so when we recall that in hemorrhage the hyperpnea is associated with a decreased hydrogen ion concentration, a decreased carbon dioxide tension and an increased oxygen tension of the arterial blood. The subsidence of ventilation upon reinjection is associated with increased acidity, increased carbon dioxide tension and decreased oxygen tension of the arterial blood. Apparently pulmonary ventilation is not accounted for by changes in the composition of the arterial blood with respect to the three primary respiratory stimulants but is in some way linked with the metabolism of the respiratory center itself which seems to be profoundly affected by changes in the volume-flow of blood (1). If the center is affected as direct experiments show other tissues (2),(3) to be affected—it is obvious that a study of the control of pulmonary ventilation is incomplete without a study of the volume-flow of blood.

The chemical control of pulmonary ventilation must in the main be looked upon as a problem in oxidations in the body as a whole and in the respiratory center as well coupled with the factors by which these oxida-

tions are maintained and by which they are disturbed. The main essentials for a normal rate of oxidation are the maintenance of an adequate supply of oxygen and the maintenance of a compatible acidity of the tissues. These two conditions are largely dependent upon the movement of the blood and the behavior of the blood as a carrier of oxygen and acid. The problem of respiration is essentially a problem in transportation of acid and oxygen between the capillaries of the lesser and greater circulation,—in other words, volume-flow of blood and the coördination of the dual function of hemoglobin. It was to follow the transportation phase of respiration and to study the regulation of circulation which is inseparably connected with respiration that the thermoelectric electric method of recording the volume-flow of blood was devised.

Continuity of results is most desirable for the study of circulation and respiration. It not only enormously multiplies the data obtainable but supplies the kind of data which is most valuable for analyzing mechanisms of control. For example, a record of the progressive changes in volume-flow of blood during a disturbance in equilibrium produced by successive administration of room air, rarefied air and room air supplies infinitely more information than a single determination of flow with the animal in equilibrium with room air and rarefied air. If such records are supported by other continuous methods of approach for following blood pressure, pulmonary ventilation, basal metabolism, blood acidity, expired carbon dioxide, oxygen, etc., a fairly comprehensive picture of some of the more fundamental occurrences related to respiration and circulation is obtained.

Important as the circulation of the blood is to an understanding of the energy exchanges of the body the methods available for a thorough study of the subject hardly meet the needs. To be sure there are numerous discontinuous methods for man and mammals which have provided valuable information but there are no suitable continuous methods which reveal the changes in circulation at the time of the experiment. The automatic and bloodless method of recording the volume-flow of blood described by one of us (4), which can be used continuously for hours, unfortunately is only adapted for recording the flow of blood through a relatively small amount of tissue such as the submaxillary gland. The need of data on the flow of blood through larger arteries such as the carotid and femoral was met by the method described below.

The method is based on the principles of the conduction of heat between one circulating fluid and another. It was assumed that other conditions remaining constant the amount of heat lost by the circulating blood flowing through a glass tube would vary in a definite way with changes in volume-flow. A sensitive thermopile placed in the course of a constant flow of water exposed to the heat of the blood responds continuously to changes in temperature of the water consequent on changes in volume-flow

of blood. These changes are recorded potentiometrically on smoked paper with the method used for recording changes in acidity of the circulating blood and body fluids (5). The type of thermopile vessel which was used for exposure of the blood is shown in figures 1 and 2 and the essential arrangements for the control of this exposure are shown schematically in figure 4.

The thermopile vessel consists in brief of a heat insulated water jacket, *A*, constructed of formica with a central glass tube, *B*, one end of which is connected by cannula with the central end of the artery, *C*, and the other with the peripheral end, *D*. The central cannula is blown to take a small thermometer for registering the temperature of the inflowing blood. The water jacket proper is made of two concentric tubes of formica with the intervening insulating space loosely packed with cotton. The blood flows at its normal rate through the water jacket in the direction indicated. Distilled water at a constant flow and constant temperature enters the lower end of the water jacket at *E* and leaves at the upper end at *F*. The water warms progressively as it flows through the jacket. The change

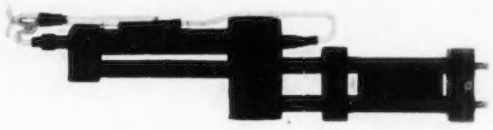

Fig. 1. Photograph of thermo-electric volume-flow recorder

in temperature of the water is detected by the thermopile, *G*, mounted in two parallel tubes, *H* and *I*. These tubes connect with the inflow and outflow tubes, *J* and *K*, of the water jacket by means of a loose friction fit. The cold junctions are exposed to the inflow water and the hot junction to the outflow water. An increase in the difference in temperature between the inflow and outflow water produces increased E.M.F. recorded by the potentiometer as an upstroke on the record. The connections with the potentiometer are made by means of binding posts, *L* and *M*. The thermopile vessel is held in place by a removable rod screwed into the upper heavy block of formica.

The thermopile proper is of the Wilson and Epps type (6). The design which we have developed for our needs is made of no. 40 Advance wire (copper nickel alloy) wound on a rectangular piece of formica about 1.5 mm. thick with 80 turns to the inch. The arrangement for plating with silver is shown in figure 3. Cathode connection with each turn of wire on the upper flat surface of the thermopile is obtained with a mercury trough constructed of paraffin extending over the windings. This insures

because of eliminating potential drop, equal plating of the half turns not covered by the paraffin and immersed in the silver cyanide solution. On plating a series of junctions between the Advance wire and the silver sheathing is thus formed along either edge of the formica strip. The formica strip with the flat surface in the horizontal position is carefully lowered into the plating solution and supported with all of the exposed wire in the solution. A rectangular plate of silver at the bottom of the bath below the thermopile serves as the anode. The half turns immersed in the bath are plated with silver to about one-third the cross sectional area of the Advance wire, and the thermopiles are then calibrated for sensitivity. Several thermopiles were made and the computation for proper plating and the sensitivity per couple is given below[1] for one of them. A similar type of thermopile with the wire wound on tubing for recording changes in body temperature is shown in figure 4.

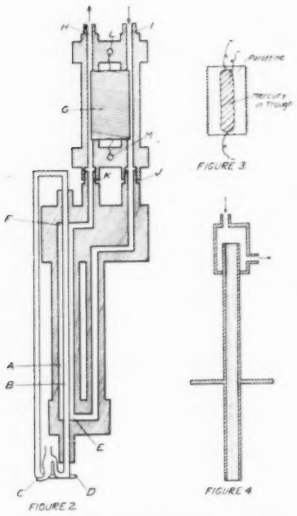

FIGURE 2.



FIGURE 3.


FIGURE 4.

Fig. 2. Scale drawing of thermoelectric volume-flow recorder.

Fig. 3. Arrangement for plating thermopile showing mercury trough and paraffin coating.

Fig. 4. Body temperature thermopile. The upper junctions are covered with a constant temperature water jacket.

The general schema of the method showing the essential arrangements for the exposure of the blood and for recording the changes in volume-flow are found in figure 5. The conditions necessary for quantitative data are the delivery of water to the thermopile vessel at a constant rate and constant temperature and thorough insulation of the thermopile vessel. The rate of flow is determined by the head of pressure and the resistance offered to the flow. A Mariotte bottle serving as the water reservoir insures a uniform pressure. The head of pressure is controlled by adjusting the height of the outflow tube. This is made of glass tubing

[1] Thermopile no. 5: 105 turns of no. 40 Advance wire (dia. = 0.00787 cm.) on formica strip 5.5 cm. wide and 3.5 cm. long.

Area of wire = $\overline{0.00393}^2 \times 3.1416 = 0.00004864$ sq. cm

Length plated = $5.5 \times 105 = 577.5$ cm.

The ratio of cross sectional area of the Advance wire to that of the silver should be not more than 3/1 (6). Wilson and Epps kept the mass of the plating to this value due to the small amount of energy available in radiometric work. Such limitations are not present in our problem and plating slightly greater than this is advantageous.

annealed at the free end to a fine opening. Inasmuch as the tubing of the water system is large the main resistance is offered by the outflow tube. If the outflow tube is kept free of small particles which may occasionally lodge at the opening and if distilled water is used there is no difficulty in maintaining a constant flow of water for hours.

Delivery of water at a constant temperature requires several precautions. Distilled water equilibrated to room temperature is used. This

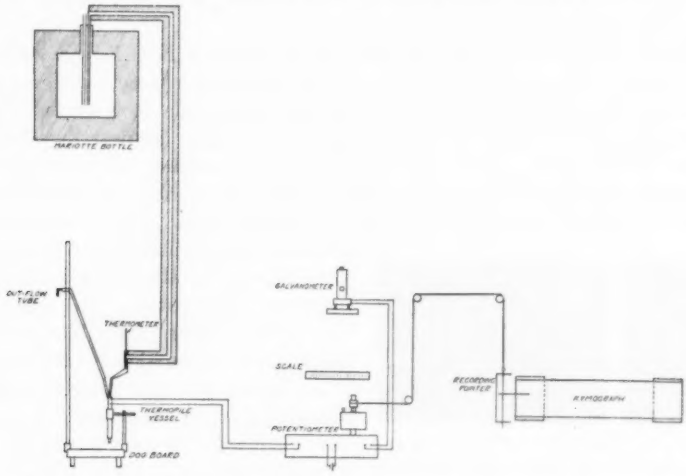


Fig. 5. General schema of thermo-electric method of recording volume-flow of blood.

keeps the temperature gradient between the water and exterior at a minimum with minor fluctuations in room temperature. The large capacity of the reservoir (40 liters) and the insulation of the reservoir and its delivery tube also give protection against rapid changes in temperature. The Mariotte bottle is placed in a large wooden box lined with paper and

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Minimum cross sectional area of silver coating $= \frac{0.00004864}{3} = 0.0000162$ sq. cm.

Mass of silver to be deposited $= 0.00001621 \times 577.5 \times 10.5 = 0.0982933$ G.

Coulombs required $= \frac{0.0982933}{0.001118} = 87.9$ or 0.008 amp. for 3 hours.

Resistance of windings before plating = 1220 ohms.
Resistance of windings after plating = 654 ohms.
With one series of junctions in water at a temperature of 0.3°C. and other series in air at 22.0°C. the E.M.F. developed was 0.00291 volt per degree Centigrade, or 0.000027 volt per degree per junction.

filled with cotton. The delivery tube is lagged with a thick layer of cotton and an outer glass tube. With this arrangement the temperature of the inflow water remains constant to one-tenth of a degree centigrade throughout the course of an experiment despite fluctuations in room temperature of several degrees centigrade. The low heat conductivity of formica and the heavy material used insure ample protection in the thermopile vessel. The constant E.M.F. in the direct experiment during fluctuation in room temperature gives the final proof that ample insulation has been achieved.

Other things remaining constant, an increase in E.M.F. indicates an increase in volume-flow of blood and a decrease the reverse, but for quantitative determinations calibration of the thermopile is essential. The thermopile is calibrated by delivering the water flow, as described above, with one Mariotte bottle and the blood with another Mariotte system carefully insulated against heat loss. The flow of blood is varied by a series of outflow tubes with openings of different sizes. The temperature of the blood is determined by a thermometer mounted in the inflow cannula of the thermopile vessel. The temperature of the water jacket flow at the inflow tube is similarly determined. These values give the maximum temperature gradient obtaining. A record of the behavior of the thermopile vessel with varying flows of blood at a constant temperature is shown in figure 6. Such records supply the data for calibration curves (see fig. 7), in which the E.M.F. is plotted on the ordinates against the volume-flow of blood on the abscissas.

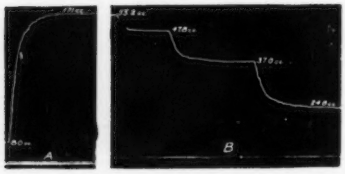

Fig. 6. Calibration record of the response of the thermopile vessel to changes in blood flow given in cubic centimeters per minute.

The extent of heating of the inflow water, and therefore also the change in E.M.F., is determined not only by the flow of blood but by the temperature of the blood as well. The greater the temperature gradient between the blood and the water in the surrounding water jacket the greater the E.M.F. developed per unit flow. This relation is shown in figure 7, in which three observed calibration curves with three different temperature gradients are plotted. Variations in the temperature gradients were obtained by varying the temperature of the blood keeping the temperature of the water jacket flow constant. Blood temperatures similar to those obtaining in the living animal were used. The observed curves are marked with circles at the observed E.M.F. values. A comparison of these curves with temperature gradients of 13, 11.2 and 9°C. respectively show the importance of this factor and the necessity of accurate determination of blood and water temperatures.

Since the temperature of the living animal varies with the individual animal and since it varies during the course of an acute experiment with a general tendency to a fall in temperature it is obvious that a series of calibration curves at different temperature gradients are essential for quantitative analysis of animal records. Fortunately the approximately

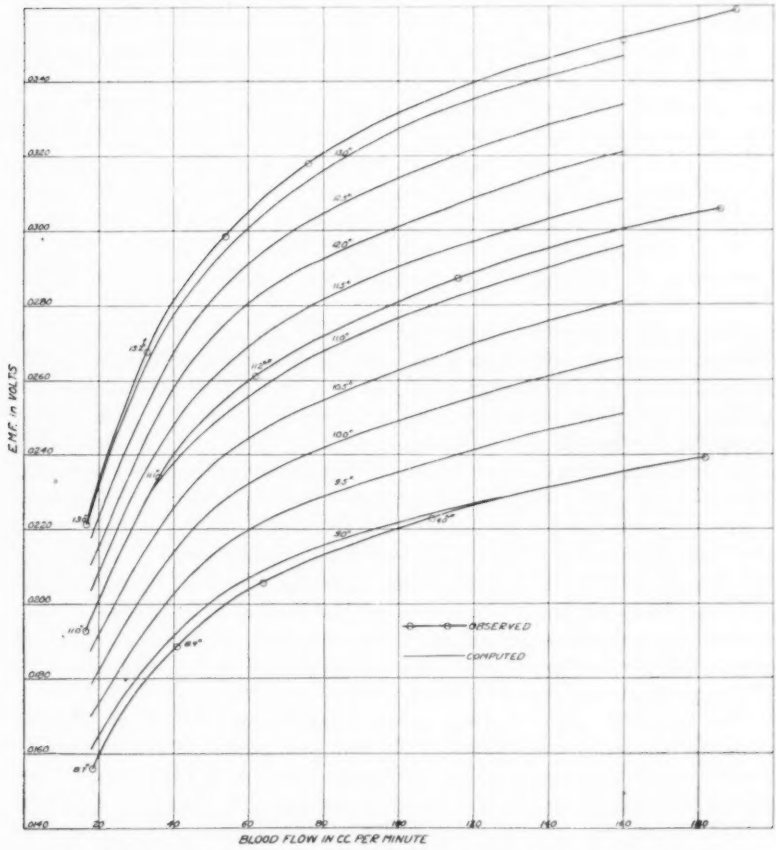


Fig. 7. Observed and computed calibration curves obtained with ox blood

linear relation of thermal conduction to the temperature gradient permits a rapid computation of the required number of curves. In figure 7 there are nine computed curves obtained by interpolation between the three observed curves. This method is justified for by using two observed curves as a basis for computation we have found computed curves which

were obtained by interpolation between observed curves (as in fig. 6) to agree with additional observed curves. A series of curves, such as are shown in figure 7, permit a rapid mental interpolation of the observed E.M.F. in terms of volume-flow.

With the use of the smoked records and the plotted curves, such as in figures 6 and 7, the thermopile vessel may be analyzed for rapidity of response, sensitivity and accuracy. The latent period of the thermopile vessel to changes in flow of blood, as determined by direct experiment, is seven seconds. The duration of this period presumably is determined by the thickness and the material of the tube conducting the blood through the water jacket and by the rate of water flow. Knowing its duration, the time relation between changes in volume-flow and other phenomena in animal experiments can be established. The rapidity of change in E.M.F. at the close of the latent period is indicated in figure 6A. Fully 75 per cent of the increased flow is registered within 26 seconds. Complete equilibrium is established more slowly but this is of relatively little significance.

The sensitivity of the thermopile vessel to changes in blood flow will vary with the characteristics of the thermopile itself, with the amount of water flow, with the amount of blood flow, and with the temperature gradient. We have varied all these factors. A slow flow of jacket water increases the sensitivity and simultaneously slows the response. We have chosen a water flow of 18 cc. per minute as suitable to our needs. This is the flow used in all the calibration curves of figure 7. The significance of the remaining factors which influence sensitivity, namely, blood flow and temperature gradient, are brought out in this figure. The slower the flow and the higher the temperature gradient the more sensitive is the vessel to changes in flow. In general—it can be said that the method may be made as sensitive as desired for animal experiments for recording changes in flow. This high sensitivity meets one of the main purposes for which the method was devised.

The accuracy of the method in recording absolute flows is also indicated by the calibration curves. For example, an E.M.F. of 0.024 volt with a temperature gradient of 9.5°C. is equivalent to 115 cc. flow, and with a temperature gradient of 9°C. is equivalent to 190 cc. An error in the determination of the temperature gradient amounting to 0.1°C. is equivalent to 15 cc. per minute or approximately 13 per cent. This error, to be sure, is large; but these particular results represent extreme conditions of low temperature gradient and high blood flow. These conditions are not met with in our animal experiments. The temperature gradients have been approximately 13°C. and the flows have usually been lower. On analyzing the accuracy of the method under more common conditions of lower flow and higher temperature gradient, the error

of 0.1°C. in the temperature gradient reduces to about 2 per cent. That is, the accuracy of the values on the calibration curves with a uniform composition of the blood flowing in a steady stream is within 2 per cent in the usual range of flow and temperature gradient obtaining in animal experiments.

If we employ the calibration curves obtained on ox blood for quantitative analysis of records in experiments on the dog the accuracy of the absolute values so determined will depend upon a number of factors, the accuracy of determining the temperature gradient, the effect of pulsatile flow, and the composition of the blood which includes such properties as specific heat and viscosity.

The temperature gradient can be as accurately determined in the animal experiment as in the in vitro experiment for the thermometer recording the temperature of the blood is in the blood stream at the same point—that is, where the blood enters the thermopile vessel. If rectal temperature is used, that might introduce an additional error of 0.1 of a degree. To bring the blood in the thermopile at body temperature the lower end connecting with the artery was carefully and thoroughly covered with loose cotton.

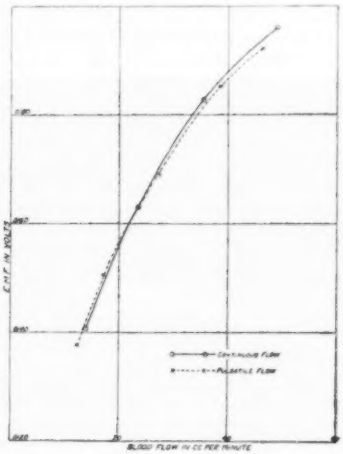

Fig. 8. Comparison of calibration curves obtained with steady and pulsatile blood flows.

The effect of pulsatile flow was studied by interrupting the flow with a rotating stopcock producing conditions approaching normal. Inasmuch as the exchange of heat between the blood and water is presumably dependent on the temperature of the blood adjoining the inside of the tube it is conceivable that a pulsatile flow might break the outer layer of blood, if such exists, and bring the warmer axial stream in closer proximity to the water. Two curves taken at random from a number of experiments in which the flow was interrupted from sixty to one hundred and eighty times per minute are shown in figure 8. Pulsation apparently does not affect the transfer of heat from the blood under the conditions which we have provided in the calibration experiments.

If this holds for animal experiments the validity of the use of the ox blood calibration curves in the animal experiment for establishing absolute flows hinges on the effects of composition of the blood. On this point we

do not have sufficient data to draw positive conclusions. Calibration curves obtained with water instead of blood showed higher E.M.F. values per given flow, which agrees with the difference in specific heat of blood and water (7). In the few experiments done on the effects of dilution we have noticed little change in heat transfer. Nevertheless, we do not care to propose the thermo-electric method as highly quantitative without further checks on the effects of dilution or concentration of blood occurring during an experiment and on the difference in composition between blood of different animals. It must be used with care if extreme accuracy is essential. On the other hand—if we are mainly interested in recording continuously changes in flow of blood with a fair degree of accuracy the method is extremely satisfactory. It meets the present needs of the study of disturbances in equilibrium in the circulatory system.

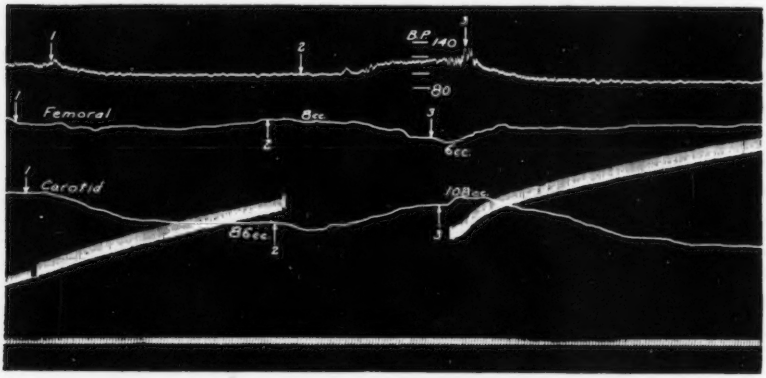


Fig. 9. Record from in vivo experiment showing *a*, the effects of increased pumonary ventilation, *b*, the administration of carbon dioxide, and *c*, readministration of room air on the flow of blood in the carotid and femoral arteries. Blood flow in cubic centimeters per minute.

A record obtained in the animal experiment in which one thermopile vessel is connected with the carotid artery and the other with the femoral artery is shown in figure 9. Twenty-one milligrams of heparin are injected intravenously as soon as the animal is ready for observation, to prevent clotting of the blood in the thermopile vessel. This amount suffices for three to five hours. All dissection is done with thermocautery to minimize bleeding from the smaller blood vessels. Mean blood pressure is recorded and pulmonary ventilation is administered and recorded. The record shows the change of flow in two large arteries with an increase in pulmonary ventilation during the administration of room air. The carotid flow diminished and the femoral flow increased. At arrow 2 carbon dioxide was administered and the reverse changes occurred. There

was an increase in flow through the carotid artery and a decrease through the femoral. Recovery on the administration of room air was accompanied by decreased carotid flow and increased femoral flow. The absolute values are based on ox blood calibration curves and are given only tentatively, as calibration curves on dog's blood have not been established. The record indicates the possibilities of studying the mechanism of blood flow control.

## SUMMARY

The need of developing a continuous method of recording the volume-flow of blood for the study of disturbances in circulatory equilibria was pointed out. A thermo-electric method was described.

The method was based on the principles of heat transfer between two circulating fluids of different temperatures. The circulating blood of a heparinized dog was exposed to a constant flow of water in a concentric water jacket. Changes in difference of temperature between the inflow and outflow were recorded by means of a sensitive thermopile. The resulting changes in E.M.F. were recorded on smoked paper with a potentiometer by a method previously described.

The relation of E.M.F. to flow of blood was established in in vitro experiments on ox blood for different temperature gradients between the circulating blood and circulating water.

Such calibration curves were used for analysis of records from animal experiments.

The calibration curves were steeper with the larger temperature gradients and at the lower blood flows. The relation of these points to the sensitivity and accuracy of the method were discussed.

The method was found to meet the present needs of studying circulatory control. A record from an animal experiment demonstrating the advantages of the method was shown.

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# THE REGULATION OF RESPIRATION

## VI. Continuous Electrometric Methods of Recording Changes in Expired Carbon Dioxide and Oxygen

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Variations in pulmonary ventilation presumably occur to meet the changing needs of the tissues—to provide an adequate supply of oxygen and a corresponding elimination of acid. The mechanism which the body employs to meet these needs is extremely complex; but if we can follow from moment to moment the variations in the gaseous exchange occurring in the lungs under a variety of conditions we have a new means of furthering the problem of respiratory control. We hoped to develop such procedures by devising continuous methods of recording changes in oxygen and carbon dioxide of the expired air.

The inestimable value of continuous methods has been experienced in the use of the continuous potentiometric method of recording changes in hydrogen ion concentration of the circulating blood and body fluids during acute physiological changes (1), (2). The method proved the value of studying disturbances in equilibrium as contrasted with determination of values under long established conditions of equilibrium. Take, as a single example, the significance of the hydrogen ion concentration of the arterial blood during different diets. Hasselbalch (3) found that the arterial blood of an individual equilibrated with his own alveolar carbon dioxide during a protein and during a carbohydrate diet showed the same pH. These results were widely accepted as indication of the control of respiration by the hydrogen ion concentration of the blood. But studies of acute physiological changes with the manganese dioxide electrode give graphic evidence to the contrary. Increasing pulmonary ventilation with decreasing acidity of the blood is frequently recorded.

The rapid accumulation of data proved to be another outstanding advantage. In addition the method records small and momentary changes which might be missed or misinterpreted as error inherent in discontinuous methods.

These same advantages we hoped to reap with the development of con-

tinuous methods applied to recording changes in carbon dioxide and oxygen content of expired air. The carbon dioxide content of the expired air under equilibrium conditions at high and low altitudes (low and high oxygen tension) tells little of the changes in transport of that gas between the pulmonary and systemic capillaries with the consequent changes in pulmonary ventilation, and, similarly, it offers little clue to changes in acidity of the tissues. On the other hand—if we can record changes in expired oxygen and carbon dioxide occurring with abrupt changes in oxygen tension we have information on the absorption and consumption of oxygen and on the progressive saturation or desaturation of the tissues with carbon dioxide. The continuous method cannot but indicate changes in acidity of the tissues. The second advantage of extensive data must follow if

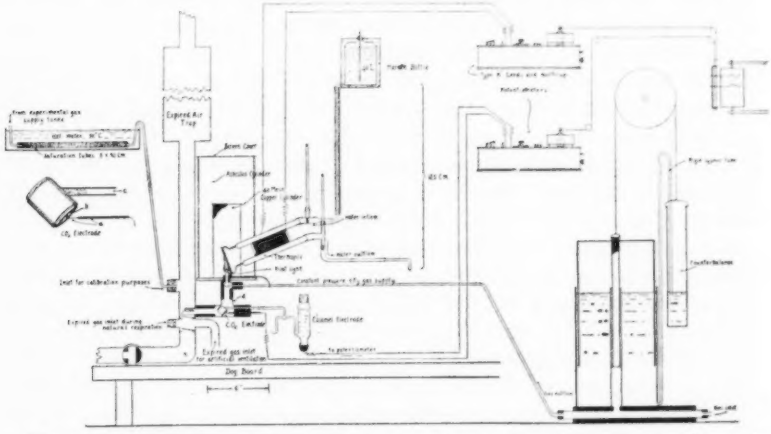

Fig. 1. Detailed schema of the continuous method for recording changes in expired carbon dioxide and oxygen. The carbon dioxide electrode and oxygen thermopile and city gas pressure regulator are drawn to scale. The reduction is indicated.

one will experiment. The time-consuming analyses are avoided and a continuous picture is unfolded synchronous with the visibly changing behavior of the animal (see figs. 16 and 17).

The fact that the acidity of a watery solution varies with the partial pressure of carbon dioxide with which it is in equilibrium was used as the basis for the registration of changes in expired carbon dioxide. The changes in acidity of a watery film exposed to the expired air were electrometrically recorded with a manganese dioxide electrode and potentiometer.

The fact that a flame of burning gas is hotter the greater the supply of oxygen was used as the basis for the registration of changes in oxygen in expired air. The records were obtained with a bunsen burner, a thermopile vessel and a potentiometer.

*In vitro experiments on the carbon dioxide method.* In general the arrangement (see fig. 1) consists of a manganese dioxide electrode, *a*, resting on a drum of peritoneal membrane, *b*, in electrolytic connection with a calomel electrode. The membrane is stretched over a short tube of formica which is telescoped into an outer cylinder of formica. It closes the opening of the side tube, *c*, which is filled with potassium chloride connecting with the calomel electrode. Moistening the membrane with potassium chloride establishes electrolytic connection between the two electrodes. An enlarged sketch of this unit is shown on the left of figure 1. We shall refer to it as the carbon dioxide electrode. This electrode is placed in a T tube or electrode vessel, *d*, in the path of the gases to be studied. The valves and other special arrangements shown in the figure are of no immediate concern. Attention is called here only to the inlet (marked inlet for calibration purposes) through which the gases are conducted in the in vitro experiment and in the animal experiment for calibration. The manganese dioxide and calomel electrodes are connected with a type K Leeds and Northrup potentiometer. Increase in acidity at the manganese dioxide electrode leads to increased E.M.F. The changing E.M.F. is kept in balance with the potentiometer and is mechanically recorded as previously described in the continuous electrometric method of recording changes in acidity of the circulating blood and body fluids (1).

An ideal carbon dioxide electrode should respond quickly to changes in carbon dioxide pressure. It should possess durability and yield absolute values interpretable in terms of carbon dioxide pressure. Some of the characteristics of the electrode are shown in figures 2, 3, 4, 5, 6, 7 and 8. Figures 2, 3 and 4 are graphic records on smoked paper of the response of various electrodes to gaseous mixtures with varying pressures of carbon dioxide, and figures 5, 6, 7 and 8 show the relation of E.M.F. developed to the percentage of carbon dioxide.

Eight gaseous mixtures of carbon dioxide in room air were used to study the carbon dioxide electrode: 15, 10, 8.5, 7, 6, 4, 2, 1 and 0 per cent. This number provided sufficient points to establish fairly accurate curves showing the behavior of the carbon dioxide electrode to changing carbon dioxide pressure. The mixtures were prepared according to Boyle's law and stored under pressure in boiler tanks of eighty gallons capacity each. Commercial carbon dioxide and compressed air were added to the tanks in proper proportions with the aid of U-shaped mercury manometers capable of recording 3500 mm. mercury pressure.

Since moisture is essential for the development of acidity and electrolytic conduction it is necessary to saturate the gases with water vapor to prevent drying of the carbon dioxide electrode. This was accomplished by delivering the gas through individual saturation tubes (5 × 90 cm.) filled with moistened coke. Saturation was facilitated by immersing the tubes in a water bath held at 50°C. (see fig. 1).

For recording the behavior of the electrode gas was introduced into the electrode vessel to quickly replace the previous gas and was then reduced to a flow barely perceptible to the lips. Two to four electrodes were studied simultaneously in the same gaseous mixture delivered through the electrode vessels (large glass T-tubes) connected in series with rubber tubing. This permits a satisfactory means of comparing the behavior of different electrodes under varying conditions.

The study of the variables which influence the behavior of the electrode is still incomplete and the method in its present state is not strictly quantitative. With the hope of making it suitable for quantitative studies in metabolism we are continuing the in vitro experiments. Several factors such as maintenance of a proper degree of moisture, maintenance of uniform concentration of potassium chloride at the electrode, size and thickness of membrane, size and plating of the electrode, buffering of the electrode, etc., need careful study. But in the meantime the method is described as it stands, for checked as it is by calibration it has proven exceedingly valuable in our experiments on the dog. The method should open new fields in biological investigations.

Three varieties of manganese dioxide electrodes were used. The first electrodes were identical to the electrodes used for recording changes in acidity of the circulating blood (no. 24 platinum wire about 2 mm. long). These were first plated with platinum black, then heated and lightly plated with manganese dioxide. Only the point of the electrode was in contact with the membrane. On the whole this type of electrode gave very satisfactory results. Its durability, however, was only fair and it required rather fine adjustment on the membrane. A longer electrode (15 mm.) of more flexible wire (no. 36) with several mm. contact was an improvement in both respects. The last electrode adopted is only 0.001 of an inch thick (no. 49) and is arranged in a small loop about 2 mm. diameter in the center of the membrane. It is exceedingly flexible which prevents the loss of plating from scraping on the membrane, and possesses the advantage of being held in place by the moisture on the membrane. These electrodes have shown excellent durability. Though the plating is exceedingly thin, of a light golden color, the electrodes appear unchanged at the end of a day's use, and behave very well on succeeding days. The standard electrodes which we used were plated in a 0.2 molar manganese sulphate solution for three seconds. A 2-volt storage cell supplied the current and the electrodes were separated 25 mm.

Commercial gold beater's skin was the first membrane used. This was thoroughly washed in soap to remove the oil. It was later replaced by the thinner peritoneal membrane of the dog which seemed on the whole to increase the rapidity of response.

The rapidity of the electrode is shown in figures 2, 3 and 4. It will be

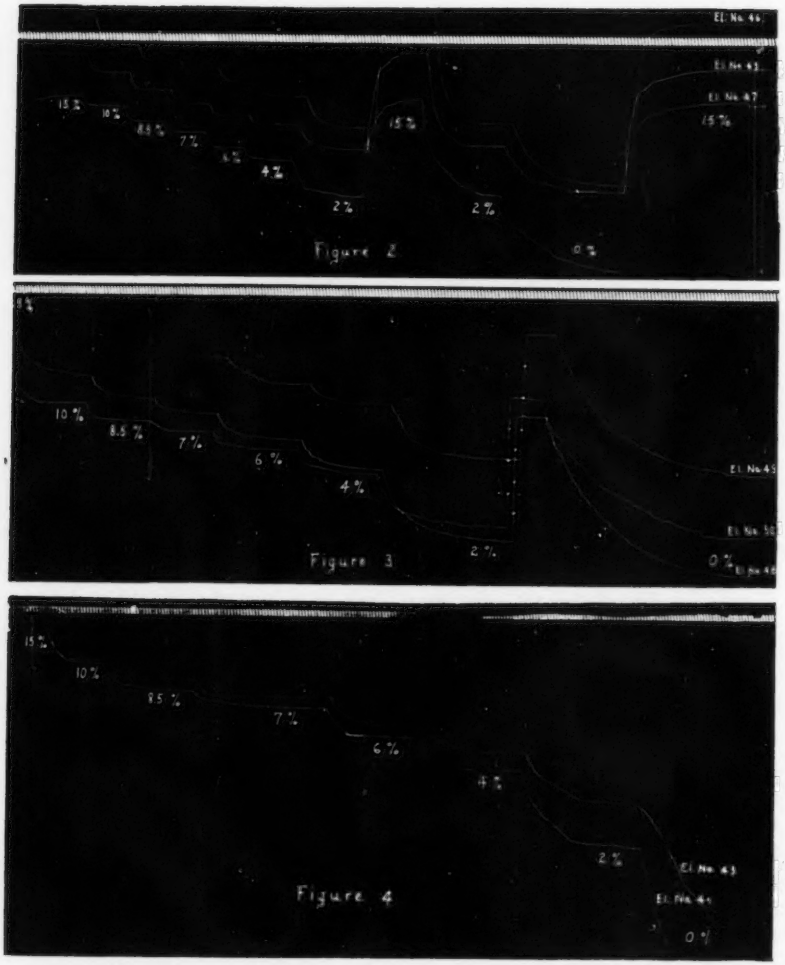


Fig. 2. Simultaneous record of the behavior of the electrodes plated three seconds to varying carbon dioxide mixtures. Electrodes 45 and 46 were freshly plated and electrode 47 was one day old. This record supplies the data for figure 8C. The percentage of carbon dioxide is indicated in the figure.

Fig. 3. Similar records of the behavior of three electrodes plated 2 seconds (no. 48), 5 seconds (no. 49), and 10 seconds (no. 50). The vertical interruption of the records to the right represents four loops up on the spindle to permit registration of the last change of gas. This record corresponds to figure 5A.

Fig. 4. Simultaneous record of two electrodes plated 4 seconds. It supplies the data for the descending curves in figure 6.

noticed that there are slight differences in behavior of different electrodes. In general the reaction is rapid—faster with high percentages of carbon dioxide (4 to 15 per cent) than with lower percentages (4 to 0 per cent). Considering the slowness of the reaction between water and carbon dioxide the establishment of equilibrium within 10 to 20 seconds, as occurred in figure 2, is very rapid. This, of course, is most desirable in animal experiments.

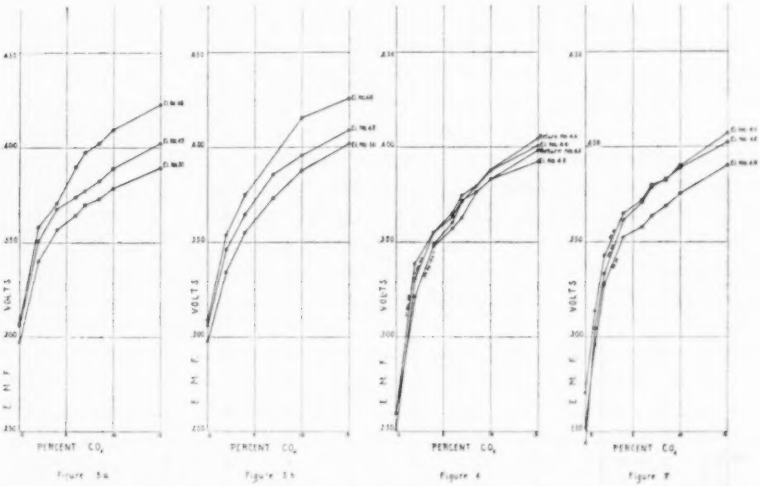


Fig. 5A. E.M.F. values corresponding to changes in carbon dioxide concentration obtained with three electrodes plated 2 seconds (no. 48), 5 seconds (no. 49), and 10 seconds (no. 50). The carbon dioxide percentage is reduced from 15 to zero (descending curves). This figure corresponds to figure 3.

Fig. 5B. Same as figure 5A but plotted with increasing carbon dioxide percentages (ascending curves).

Fig. 6. Descending and ascending curves showing relation of E.M.F. to carbon dioxide concentration with two electrodes plated 4 seconds. This figure corresponds to figure 4.

Fig. 7. Descending curves showing relation of E.M.F. to carbon dioxide concentration with electrodes plated 1 second (no. 40), 2 seconds (no. 41), and 4 seconds (no. 42).

In regard to the rapidity of response—it should be recalled that in the pH studies of the blood the quickness of response was largely determined by the thickness of the plating. With the carbon dioxide electrode the quickness of response is probably determined not only by the quickness with which the manganese dioxide comes into equilibrium with the surrounding fluid but also by the quickness with which the surrounding fluid comes into equilibrium with the carbon dioxide. Inasmuch as the plating

is very thin on the carbon dioxide electrode that factor is minimized. At least there has been no constant difference in rapidity of electrodes plated one to five seconds or more (see fig. 3). The condition of the membrane with regard to amount of potassium chloride and moisture as well as the thickness of the membrane seem to be the important variables. In all cases the response was quick enough to register short changes in carbon dioxide content.

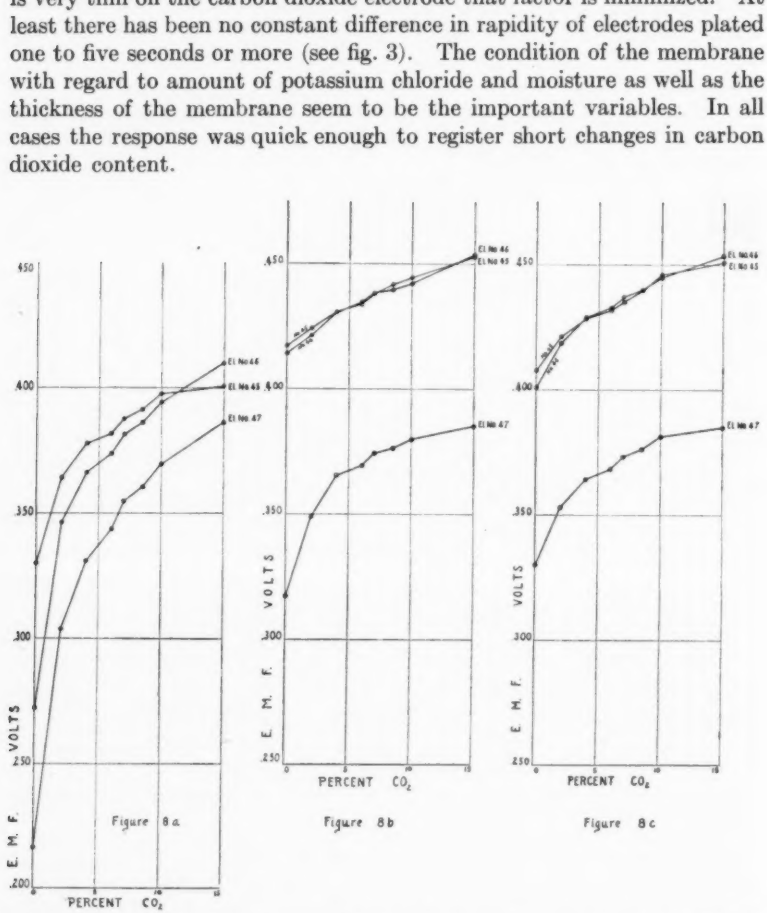


Fig. 8 A, B and C. Three successive sets of descending curves showing relation of E.M.F. to carbon dioxide concentration with electrodes used in figure 2.

The degree of reproducibility of behavior of electrodes with regard to absolute E.M.F. values with varying carbon dioxide tension is shown in figures 5, 6, 7 and 8. The data in figures 5A and 5B were obtained with three freshly plated electrodes (three seconds' plating). In figure 5A gaseous mixtures of progressively decreasing carbon dioxide concentrations were introduced into the electrode vessel. This was followed immediately by the introduction of gaseous mixtures of progressively increasing carbon

dioxide tensions—figure 5B. Considering that no buffer was used the reproducibility was very fair. In figure 6 the reproducibility of two electrodes (four seconds' plating) is still better. The response of each electrode to decreasing and increasing carbon dioxide is shown. In figure 7 the electrodes were plated one, two and four seconds. Here, too, the reproducibility is fair. But differences in absolute values may develop, as is indicated in figures 8A, B and C. In these figures the same electrodes were used—two fresh electrodes and one electrode one day old. In the first set of curves the behavior of the three electrodes is fairly similar. In the second and third sets the values between the fresh and old electrodes diverge. The changes in behavior which lead to such divergences fortunately can be checked in the course of an animal experiment by a convenient and rapid method of calibration.

Though the absolute values for any particular carbon dioxide percentage may vary from electrode to electrode, the behavior of the electrode to changes in carbon dioxide is in general relatively uniform. On the whole the electrode meets the urgent needs of our problem of recording continuously with reasonable accuracy rapid changes in expired carbon dioxide. It is these changes which may shed important light on the mechanism of respiratory control.

*In vitro experiments on the oxygen method.* The crude trial experiment of testing the heating capacity of city gas burning in a bunsen flame in a twenty-one and in an eight per cent oxygen mixture gave promise of a continuous method of recording changes in oxygen content of gaseous mixtures. The development of the method, however, required considerable experimentation. The arrangement finally adopted is shown in figure 1 along with the carbon dioxide electrode.

A constant flow of water delivered at a constant temperature through the cold junction tube of a thermopile vessel is exposed in a small copper tube to the heat of the bunsen flame. The heated water continues through the hot junction tube of the thermopile vessel and leaves the water system at the end of the outflow tube. The changes in E.M.F. resulting from changes in difference in temperature between the cold and hot junctions, due to variations in the oxygen supply of the flame, are balanced with a type K, Leeds and Northrup potentiometer and mechanically recorded on smoked paper.

A constancy of flow of water is provided with a Mariotte bottle. The rate of flow is adjusted by the head of pressure and the total resistance offered to the flow. The resistance is controlled by the size of the opening in the outflow tube, which is annealed to suit the needs of the experiment. In our arrangement the driving head of pressure was 125 cm., and the flow of water 103 cc. per minute. The exclusive use of distilled water and an outflow tube of large capacity yielded a constant flow of water from day to

day. The water was delivered to the thermopile at a constant temperature by means of a Mariotte bottle (40 liters capacity) filled with water at room temperature insulated against heat transfer. The water was conducted directly to the thermopile in well insulated tubes. A thermometer in the course of the stream at the cold junction registered a constant temperature despite large fluctuations in room temperature.

The thermopile proper is of the Wilson and Epps (5) type of the design described in the continuous method of recording the volume flow of blood (6). It consists of three hundred turns of no. 40 Advance wire wound 80 turns to the inch on a card of formica of the dimensions indicated in figure 1. One-half of each loop is plated with a sheath of silver, making three hundred cold junctions at one edge of the card and three hundred hot junctions at the other. Inasmuch as the heat energy of the system is large the effect of conduction of heat by the silver away from the water is small, and a relatively heavy coating of silver is without harm. The thermopile is cemented in slits of heavy formica tubing with the hot and cold junctions protruding into the water stream approximately 1.2 mm. The thermopile is tested or calibrated by placing the copper tube in the bunsen flame with the water running at a constant rate and noting the difference in temperature of the inflowing and outflowing water and the E.M.F. developed. It is advisable to dry the thermopile vessel after experiments to prevent local chemical action at the junctions. The absolute E.M.F. values and the total electrical resistance serve as checks on the condition of the thermopile.

The control of the bunsen burner and the means of absorbing the heat of the flame are shown in scale drawing in figure 1. The first essentials are the proper supply of the oxygen mixture and of the combustible gas. The supply of the oxygen mixture to the bunsen flame is self-regulating. It is determined by the dimensions of the burner and the draft created by the heat of the flame. With high percentages of oxygen the flow of the oxygen mixture is increased. As a result the consumption of oxygen by the flame is further increased. Such changes in the utilization of oxygen, however, do not affect the accuracy of the method as they are accounted for in calibration. The first important factor, therefore, is a constant supply of heat units. As will be shown later, the composition of city gas is uniform enough for accurate observations, but the fluctuations in pressure require regulation. This was accomplished with an automatically balanced Hutchinson spirometer illustrated in figure 1, also drawn to scale. The spirometer is constructed of formica tubing of uniform bore and thickness of wall. The inverted tube is nicely balanced in the empty position by a weighted brass tube suspended over a freely running pulley. The cross sectional area of the opening of the tube is exactly equal to the cross sectional area of the wall of the inverted cylinder of the spirometer. The

brass tube is provided with a syphon tube connected with the water jacket of the spirometer. If the inverted tube rises as a result of excess of inflow of gas from the city main over outflow to the bunsen burner the buoyant force of the water is diminished and the pressure in the spirometer tends to rise. But a rise in the cylinder is accompanied by an equal fall in the brass tube counterweight. Water flows into the counterweight in proportion to the decrease in buoyant force and the pressure within the spirometer remains constant. To avoid the disturbing effects of a change in level of the water jacket consequent on the contribution of water to the counterbalance a reservoir of proper capacity was connected by tube to the water jacket to increase the available volume. Three smoothly rounded wire loops serving as a guide on the inside tube of the water jacket kept the gas container in vertical position and free of gross friction. As a matter of convenience two electrical contacts were mounted on the wheel to signal the full and empty positions of the spirometer, and a slight adjustment of the inflow cock corrected the movement of the spirometer. The slowness of the movements of the spirometer permit precise regulation of pressure. The experimental data show this to be true.

Another essential in the oxygen method is the shielding of the flame from drafts. Air currents not only vary the outside supply of oxygen from the room air to the flame but move the flame with respect to the water flow tube. The shielding was accomplished with an inside 40 mesh copper cylinder and an outside asbestos cylinder mounted on a wooden base. Each cylinder was closed above with a copper screen. The openings on the side were only large enough to admit the tubes of the thermopile vessel. Even with this arrangement strong drafts in the room were felt by the flame, but precautions against the ordinary movements of air in the room insured a steadily burning flame.

The next essential is a satisfactory utilization of the heat of the flame with varying oxygen supplies. The change in shape, position and hotness of the flame required considerable adjustment before a suitable relation between heat absorption (E.M.F.) and oxygen supply was obtained. Our wish was to secure a linear relation between oxygen content of the gaseous mixture and E.M.F. developed. The water tube placed horizontally at any level failed even roughly to approximate these relations. The arrangement shown in figure 1 so nearly gave the optimum relation (see fig. 9A) that it was finally accepted. A no. 24 copper sheet 2.5 cm. wide is soldered to the copper tube and placed in the position and angle indicated. The copper sheet absorbs and carries the heat freely to the copper tube. It is wider than the flame and therefore small movements of the flame have little effect on the amount of heat absorbed. Also the heat capacity of the sheet of copper presumably buffers temporary fluctuations in the flame.

One more precaution should be mentioned; namely, against the forma-

tion of free gas in the water as it passes through the flame. Small bubbles are likely to accumulate along the thermopile and give rise to distorted E.M.F. levels or to rapid fluctuations in E.M.F. With the water at a room temperature of 22°C. no difficulty is encountered, but if the water is

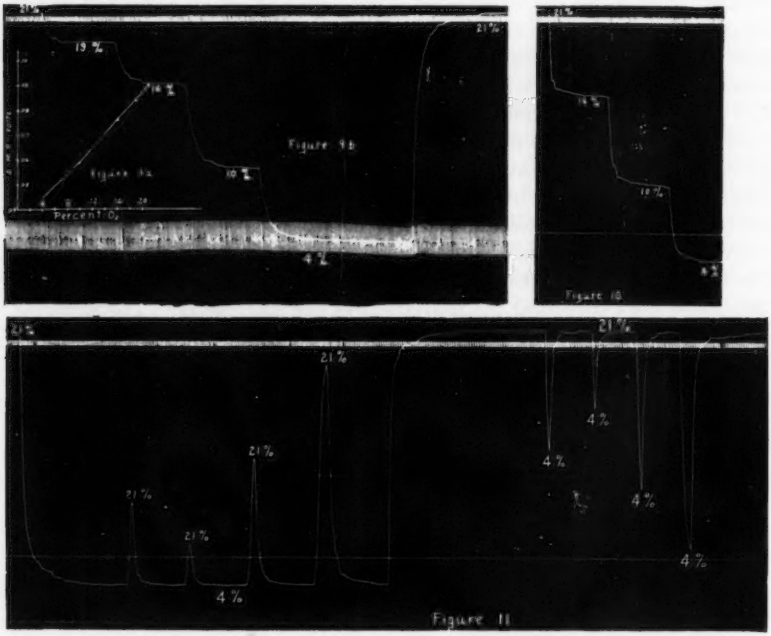


Fig. 9A. E.M.F. generated by oxygen thermopile plotted against oxygen percentage of the gas delivered to the bunsen burner. The data for this curve are obtained from figure 9B.

Fig. 9B. Record showing the response of the oxygen thermopile to successive reductions in oxygen percentage of the gas delivered to the bunsen burner. The oxygen mixtures are delivered from the experimental gas delivery tanks through the pump and artificial lungs. The curves therefore represent the lag of the ventilating system plus the lag of the oxygen thermopile. These curves are valuable for the interpretation of animal experiments.

Fig. 10. Response of the oxygen thermopile to changes in oxygen mixtures when these mixtures are delivered directly to the bunsen burner. The lag of the ventilating system is missing.

Fig. 11. Response of oxygen thermopile to large and rapid fluctuations in oxygen.

delivered at 25°C. the additional increase of 10°C. temperature on passing through the flame produces bubbles. We have had this particular difficulty only once. It, of course, could be eliminated by cooling the water or by increasing the flow of water. With the latter arrangement it might

be advisable to use a more sensitive thermopile. Our main precaution was to use freshly distilled water which is relatively free of dissolved gases.

The behavior of the oxygen thermopile to changes in oxygen tension of a gaseous mixture is shown in smoked paper records in figures 9, 10 and 11. In figure 9B oxygen mixtures of 21, 19, 16, 10, 4 and 21 per cent were successively administered. On changing from 21 per cent to 4 per cent oxygen there was a drop in potential from 0.91 of a volt to 0.45 of a volt. This represents four turns of the drum of the potentiometer, which is a convenient movement for manual balancing of the current. Attention is called to the exact return of the original E.M.F. on the readministration of the 21 per cent oxygen mixture. This is characteristic of the oxygen thermopile. It is precise and dependable and this despite the fact that ordinary city gas is used. There may be slight variations in absolute E.M.F. values from day to day but on the same day they have been constant. Presumably the day-to-day variations are due in part to changes in composition of the gas—it is rated at 550–575 B.T.U. per cubic foot. The day-to-day variations in no way interfere with the method for it is only a matter of a few minutes to calibrate and this is done as a routine procedure at the beginning and end of an experiment. It might be mentioned that up to the present city gas has proven more satisfactory than hydrogen.

It will be noted that the rapidity of response varies in figures 9B, 10 and 11. The initial response to a change in gas is invariably rapid but equilibrium is more slowly established in figure 9B. This difference is a function of the delivery of the gas rather than of the thermopile itself. In figure 9B the gases are delivered through the respiratory pump, its connecting tubes and artificial lungs to simulate conditions of the animal experiment. In figure 10 the gases are delivered directly to the burner. These figures show that by far the greatest part of the change is recorded within five seconds. Figure 11 is added to illustrate further the speed of the oxygen thermopile in recording large changes in oxygen pressure.

The oxygen method should be adapted to accurate metabolism experiments. This phase of the subject is yet to be developed. Our main interest at present is to study phenomena accompanying disturbances in equilibrium and for these studies nothing is gained in making the usual gasometric corrections. The strictly quantitative phase is reserved for later study.

It might be mentioned that a modification is being developed in a discontinuous and in the continuous method to replace the thermopile with a bolometer—the platinum wire being placed directly in the flame. This is done in the continuous method not for improving the behavior of the instrument but for securing a more compact and convenient apparatus for general use.

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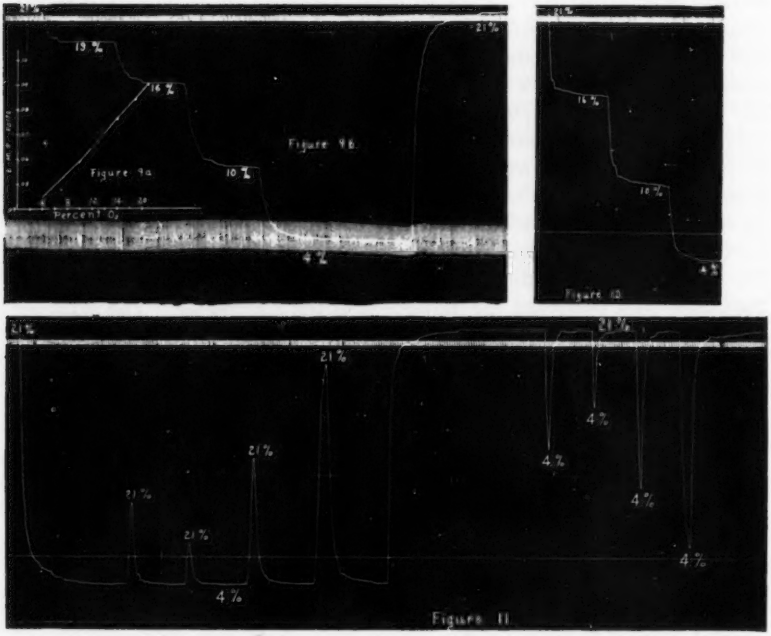


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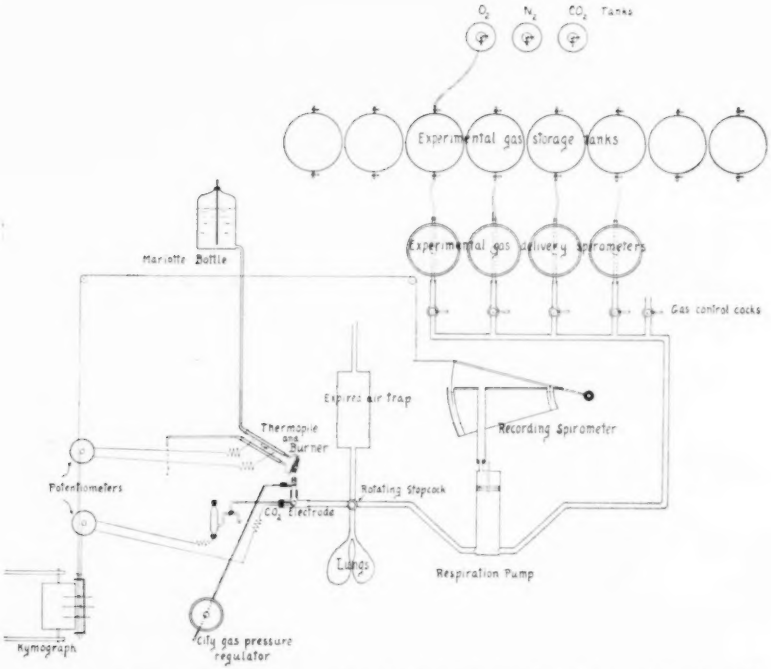


Fig. 12. General schema of apparatus for in vitro and in vivo experiments with the carbon dioxide and oxygen methods.

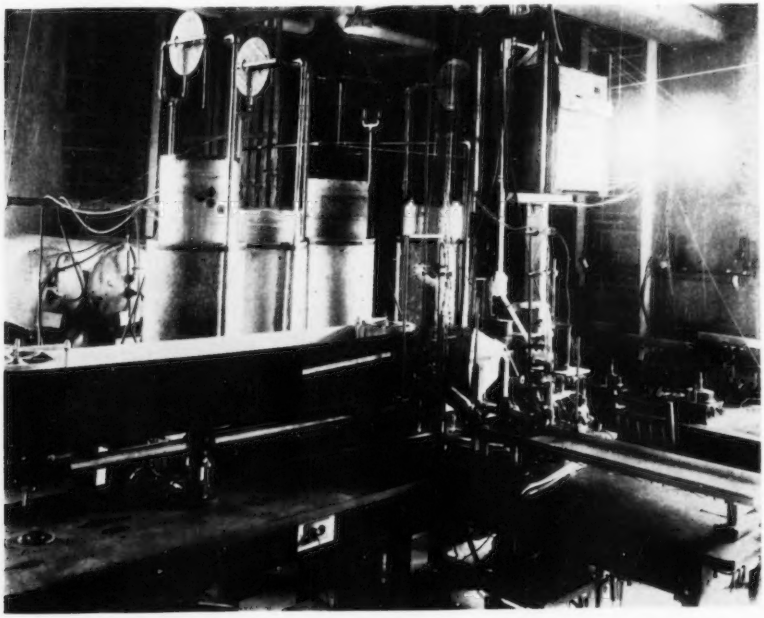

Fig. 13. Photograph showing detailed arrangement of apparatus

*In vivo experiments.* Two methods of experimentation are open. One in which the animal is permitted to breathe as it will, and the other in which pulmonary ventilation is controlled by artificial means. The first tells of the composition of the alveolar air, the gaseous pressures to which the pulmonary circulation is exposed. If pulmonary ventilation is measured it tells the total amount of oxygen absorbed and carbon dioxide eliminated as well. But in this respect the second method is considerably simpler. Since it eliminates changing ventilation the carbon dioxide and oxygen curves are directly interpretable in terms of oxygen taken up and carbon

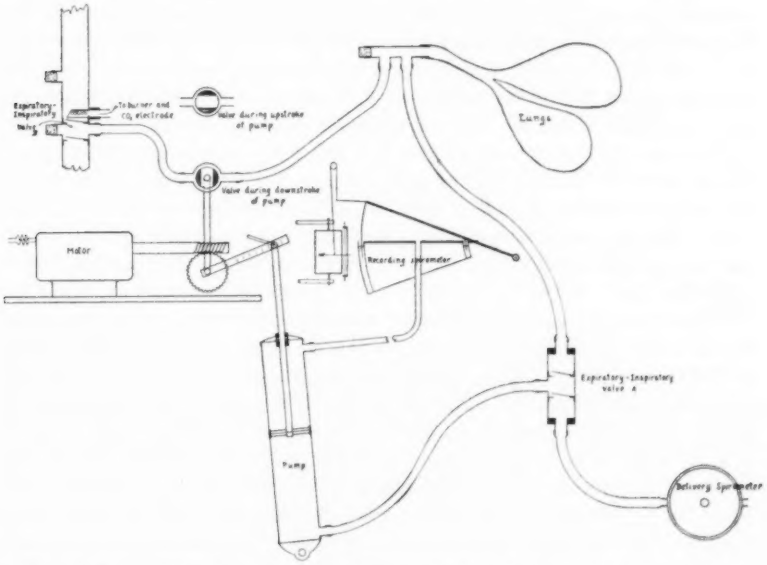


Fig. 14. Schema showing directional movement of air during artificial ventilation in animal experiments.

dioxide given off. The advantages of both methods are obvious. Up to the present the second has been the more productive of crucial data for our problem and is therefore described first.

The arrangement is outlined in figure 12 (see also figs. 1, 13 and 14). Commercial oxygen, nitrogen and carbon dioxide and compressed air were used to make up the experimental gas and calibration mixtures which are stored in the experimental gas storage tanks. The gas is delivered from these tanks to large Hutchinson spirometers (experimental gas delivery spirometers). These spirometers deliver the gas at atmospheric pressure to the respiration pump which ventilates the animal. With each

double stroke of the piston air is drawn from any chosen spirometer and forced into the lungs. At the proper time the rotating cock opens the lungs collapse and the air is expired through the expired air trap into the room. The dead end of the pump is connected with a spirometer which records the magnitude of the stroke. The carbon dioxide electrode and oxygen thermopile are shown to the left connected with the recording potentiometers. A photograph of the set-up shows the detail (see fig. 13).

As the success of these experiments hinges on the precision with which ventilation is supplied, a more accurate schema is presented in figure 14, giving the mechanism of the directional movement of the air. A single cylinder piston pump provided with a valveless double leather piston forces the gaseous mixture from the delivery tank into the lungs. The inspiratory and expiratory valves A and B direct the movement of the gas. On the upstroke of the piston the upper valve closes, the lower opens (inspiratory and expiratory valve A), and the cylinder fills with the gas supply. On downstroke the lower valve closes, the upper valve opens, and the gas is forced upward towards the lungs. During this complete double stroke the cock connecting the lungs with the exterior makes one complete rotation. During the entire downstroke (180° rotation) the cock is closed and the air from the pump is forced into the lungs. During the entire upstroke (180°) the cock is open and the lungs collapse and expel the air into the room through an inspiratory and expiratory valve, B. The outlet for the expired air goes to the carbon dioxide electrode and bunsen burner of the oxygen thermopile. The stroke and the rate of the pump are adjustable. The pump is well constructed,—it is positive and smooth in its action and delivers perfect ventilation free of injury from sudden stretch of the lungs. The rate of filling of the lungs is determined solely by the stroke of the pump. The lungs, however, are free to empty as they will. This delivers the initial expired air to the electrode and bunsen burner with a puff which frequently extinguishes the flame. This difficulty was partly overcome by a series of holes in the rotating stop cock—pyramidal in arrangement—to deliver the air by increment to the bunsen burner as the cock revolved. The initial wave of pressure at the burner was further reduced by passing the gas through a perforated tube shown immediately above the expiratory valve, B. These changes help but do not suffice to completely protect the flame with the lower percentages of oxygen. A small pilot burner burning in room air overcame the difficulty. The amount of heat which the pilot flame delivers is small and in addition is corrected for in the calibration of the thermopile vessel.

The inspiratory and expiratory valve B is unessential in the artificial ventilation experiments as the tube below the inspiratory valve is permanently closed. It is necessary, however, for recording normal ventilation, and its action in that connection will be described below. The ex-

pired air trap above the valves is used in both types of experiments. It serves as a supply of normal expired air to the burner and electrode over a long period of mechanical asphyxia. On resumption of breathing the oxygen thermopile and the carbon dioxide electrode are in equilibrium with the expired air preceding mechanical asphyxia and are ready to immediately record changes in expired air resulting from the mechanical asphyxia.

In the experiments in which the animal controls its pulmonary ventilation a specially devised recording spirometer is used (see fig. 15). It is the same spirometer used in the artificial ventilation experiments but during normally controlled ventilation it is automatically operated by weight and solenoid. The spirometer is so weighted that it will fill when

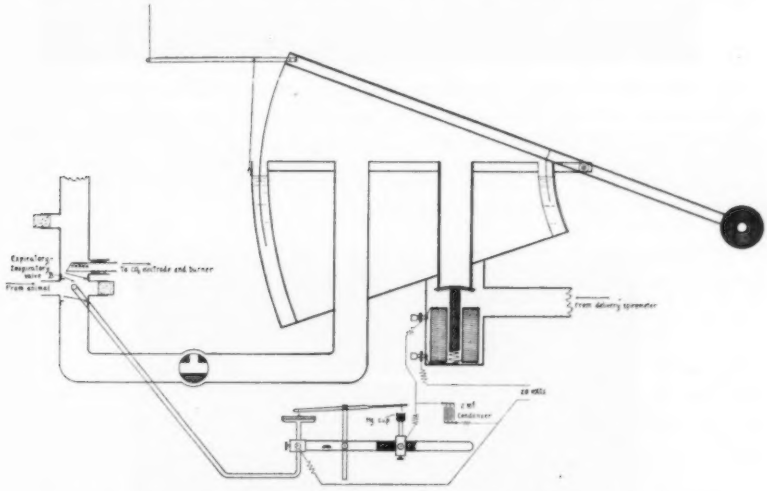

Fig. 15. Automatically filling spirometer for recording normal ventilation

the valves are open. The movement of air is controlled by the inspiratory and expiratory valve B connected with the trachea of the animal and the valve operated by the solenoid. At the end of expiration the spirometer is full of air. The intake or solenoid valve is closed and the expiratory valve has closed. On inspiration the air in the spirometer is inspired and on expiration expelled to the exterior through the expired air trap. During expiration the air in the spirometer is automatically replaced. A tambour connected with the inspiratory and expiratory valve closes the electrical contact which energizes the solenoid. The intake valve of the spirometer is drawn down and the spirometer fills to a constant point with gas from the large delivery spirometer. At the close of expiration the pressure in

the tambour falls, the contact breaks, the intake valve closes by means of a spring, and on inspiration the spirometer empties again. When the automatic spirometer is used the expired gas inlet for artificial ventilation is closed. The trachea is connected with the expired gas inlet for natural respiration and the large cock at the head of the dog board is opened.

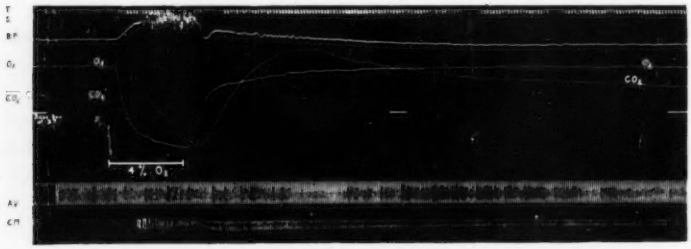

Fig. 16. Animal experiment showing the use of the continuous methods of recording changes in expired carbon dioxide and oxygen. Pulmonary ventilation is artificially maintained at a constant value. A 4 per cent oxygen mixture is substituted for room air for about two minutes. *T*, time in seconds and five seconds. *B.P.*, mean blood pressure. $O_2$, expired oxygen. $CO_2$, expired carbon dioxide. *A.V.*, artificial ventilation. *C.M.*, chest movements.

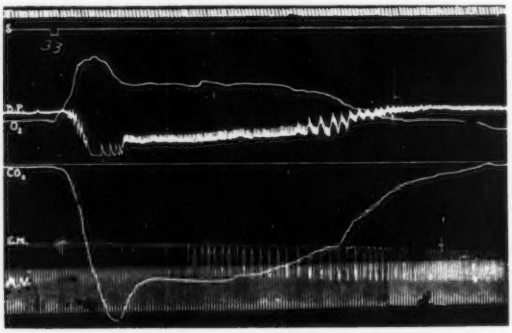

Fig. 17. Animal experiment showing the effects of sodium cyanide. *T*, time in seconds and five seconds. *B.P.*, mean blood pressure. $O_2$, expired oxygen. $CO_2$, expired carbon dioxide. *C.M.*, chest movements. *A. V.*, artificial ventilation. The rapidity of the method is indicated by the small waves in the oxygen and carbon dioxide curves synchronous with waves in blood pressure.

Results with the continuous carbon dioxide and oxygen methods in the animal experiments are shown in figures 16 and 17. In figure 16 a 4 per cent oxygen mixture is substituted during a constant artificial pulmonary ventilation with room air. The drop in expired oxygen is accompanied

by a drop in expired carbon dioxide which is the immediate effect of a disturbance in the coördination of the dual function of hemoglobin. With the low oxygen pressure the hemoglobin is reduced, alkali is thereby liberated, leading to a retention of carbon dioxide by the blood. Readministration of room air is accompanied by a corresponding increase in expired oxygen and carbon dioxide. The long continued low level of the expired oxygen curve (compare with the calibration curves in fig. 9A in which the final 21 per cent oxygen mixture is administered) indicates that the animal is consuming oxygen in excess of normal, presumably to compensate for the preceding period of deficiency. Corresponding with this increased oxygen use the formation of carbon dioxide is most probably increased. The recorded increase in expired carbon dioxide may, therefore, be accounted for both by the elimination of the retained carbon dioxide which is now free to leave due to the oxidation of the hemoglobin, and to the excess formation of carbon dioxide in the tissues. This type of record indicates the value of the method in studying the chemical function of the blood and changes in tissues metabolism.

In figure 17 a sublethal injection of sodium cyanide was administered during artificial ventilation with room air. There are pronounced changes in expired carbon dioxide and oxygen associated with changes in the use of oxygen and with changes in transport of the gases associated with variations in volume-flow of blood. The sudden fall in blood pressure is accompanied by a rise in expired oxygen to about 20 per cent and a fall in expired carbon dioxide to about 1 per cent. The slight sudden increase in blood pressure is accompanied by a sudden increased oxygen absorption and a sudden increased carbon dioxide elimination. The subsequent gradual improvement of blood pressure is accompanied by a gradual convergence of the oxygen and carbon dioxide curves. The final sudden improvement of circulation is accompanied by a corresponding abrupt convergence of the curves.

## SUMMARY

The need of continuous methods of recording changes in expired carbon dioxide and oxygen for the study of disturbances in equilibrium in the absorption, elimination, and transport of these gases as contrasted with a similar study under equilibrium conditions was discussed.

The method devised for recording changes in carbon dioxide tension consisted of a fine manganese dioxide electrode resting on a peritoneal membrane moistened with potassium chloride in electrolytic connection with a calomel electrode. This unit which is called the carbon dioxide electrode was exposed to a steady flow of expired air. Increased or decreased tension of carbon dioxide led to increased or decreased acidity of the carbon dioxide electrode. The resulting changes in E.M.F. were bal-

anced with a type K Leeds and Northrup potentiometer and mechanically recorded on smoked paper.

In vitro experiments with the carbon dioxide electrode showed a fair degree of reproducibility of absolute E.M.F. values with varying carbon dioxide tension. Similar reproducibility of behavior to changes in carbon dioxide tension were obtained. A rapid means of calibration during animal experiments was described.

The method devised for recording changes in oxygen tension of the expired air consisted of a bunsen burner supplied with a constant flow of combustible gas and expired air. Changes in oxygen tension of the expired air led to changes in intensity of the heat of the flame. The effects of the flame on a constant flow of water were recorded with a sensitive thermopile. The changing E.M.F. was balanced and recorded as in the carbon dioxide method.

In vitro experiments demonstrated the accuracy and reliability of the oxygen method. A rapid means of calibrating the oxygen thermopile during an animal experiment was described.

The advantages of the method for recording expired carbon dioxide and oxygen were demonstrated by records from animal experiments.

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# THE COMPARATIVE CAPACITY OF THE BLOOD AND OF THE TISSUE TO ABSORB CARBONIC ACID

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The capacity of the blood to absorb $CO_2$ has been determined by the direct analysis of blood samples which have been brought into equilibrium with known tensions of $CO_2$, and from these data the $CO_2$ absorption curve for blood has been determined.[1] The capacity of the tissues to absorb $CO_2$ cannot be ascertained by direct analysis, since the tissue fluids cannot be extracted from the tissues without undergoing very fundamental chemical alterations. It is, therefore, necessary to resort to some indirect method of determination. Stated very briefly the object of the experiments which follow is to determine *1*, the per cent of total acid absorbed by the body which is found in the tissues, and *2*, the gradient of the $CO_2$ absorption curve for the tissues. By "tissues" we refer to the tissues of the body collectively, exclusive of the blood.

In these experiments a cat is made to breathe air enriched with $CO_2$ until the body is saturated and can take up no more. From the total volume of $CO_2$ which is absorbed in this manner by the body as a whole is deducted the total volume in the blood, the latter being determined by direct analysis of blood samples and use of a factor for blood volume. The buffering capacity of the tissues acting alone is thus made apparent. If now the $CO_2$ absorbed by the tissues is correlated with the rise in alveolar $CO_2$ tension, the gradient of the $CO_2$ absorption curve for the tissues may be determined. The whole body is thus made to act as a tonometer and the alveolar air as the equilibrating gas mixture.

TECHNIQUE. A cannula is inserted in the trachea and artificial ventilation is given throughout the experiment by means of a respiratory pump. The anesthesia consists of an intraperitoneal injection of a solution of veronal—0.3 gram per kilo weight. In order that there shall be no muscular resistance to the pump the cat is curarized.

The pump used is that designed by Drinker (1925). It is singularly efficient because of the accuracy with which it may be set to work at any rate or volume delivery desired, and because of the absolute constancy with which it maintains these conditions. Artificial respiration is used

instead of relying upon normal respiration for the following reasons: 1. The cat is slightly over-ventilated with room air before administering the $CO_2$ gas mixture, since if we desire to increase the alveolar $CO_2$ tension by 75 mm., it is better to start with an initial alveolar $CO_2$ tension of 25 mm. and increase it to 100 mm. than to start with 50 mm. and increase it to 125 mm. 2. Under the stimulus of breathing $CO_2$ rich air the minute volume of the expired air would be four or five times as great as that which is delivered by the pump. Greater accuracy can be attained in measuring the gaseous exchange if we deal with the high percentage differences of small volumes than with the low percentage differences of large volumes. 3. All fluctuations of the minute volume must be eliminated in order to maintain the respiratory quotient at a constant level. The necessity of holding the respiratory quotient constant will become apparent further on.

Figure 1 is a diagrammatic representation of the apparatus used. The gas mixture is delivered from a steel pressure cylinder which is charged

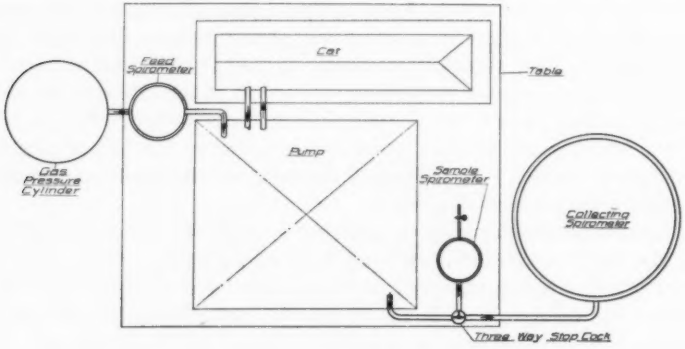


Fig. 1. Apparatus for determining $CO_2$ absorption

in the laboratory with $O_2$, $N_2$ and $CO_2$ at the concentrations desired. The gas mixture used contained approximately 7.5 per cent of $CO_2$ and 18.5 per cent of $O_2$. From this cylinder the gas is allowed to run into a feed spirometer having a capacity of about 3 liters, and from this spirometer the gas is withdrawn by the pump and delivered to the cat.

The expired air may pass directly to the collecting spirometer or it may be sidetracked into a small one-liter sampling spirometer, so that samples of expired air may be taken at intervals during the experiment. Throughout this entire system there is only one possibility for the escape of gas. $CO_2$ will diffuse through almost any fluid medium except mercury. The collecting spirometer has a water seal through which $CO_2$ will escape very slowly. This, however, can be prevented if the water in the seal is kept

saturated with $CO_2$ at approximately the same tension as the $CO_2$ in the expired air which it is intended to collect. The feed spirometer has a mercury seal, thus rendering it quite impervious to the escape of $CO_2$.

The data from a typical experiment are as follows:

*Experiment 3.* Volume of $CO_2$ absorbed by body (blood and tissue) buffers:

$O_2$ consumed $\times$ R.Q. = $CO_2$ produced in body.

$2546 \times 0.72 = 1832$ cc.

$CO_2$ produced in body $-$ $CO_2$ in expired air = $CO_2$ absorbed by body.

$1832 - 1462.4 = 369.6$ cc.

Volume of $CO_2$ absorbed by blood buffers:

Volumes per cent of $CO_2$ absorbed $\times$ Blood volume.

24.9 volumes per cent $\times$ 187 cc. = 46.6 cc.

Volume of $CO_2$ absorbed by tissue buffers:

Volume of $CO_2$ absorbed by body $-$ Volume of $CO_2$ absorbed by blood = $CO_2$ absorbed by tissues.

369.6 cc. $-$ 46.6 cc. = 323.0 cc.

$$\text{Per cent of total } CO_2 \text{ absorbed by body found in the tissues} = \frac{323.0}{369.6} = 87.4 \text{ per cent.}$$

Gradient of $CO_2$ absorption curve for blood:

$$\frac{\text{Volume per cent } CO_2 \text{ absorbed by blood}}{\text{Increase in } CO_2 \text{ tension}} = \frac{20.0}{54.9} = 0.364 \text{ volume per cent of } CO_2$$

for 1 mm. rise in alveolar $CO_2$ tension.

Gradient of $CO_2$ absorption curve for tissues:

$$\frac{\text{Volumes per cent } CO_2 \text{ absorbed by tissues}}{\text{Increase in alveolar } CO_2 \text{ tension}} = \frac{10.05}{68.4} = 0.147 \text{ volume per cent } CO_2$$

for 1 mm. rise in alveolar $CO_2$ tension.

*The volume of $CO_2$ absorbed by the body (blood and tissue) buffers.* The effect of breathing $CO_2$ rich air is to dam back the $CO_2$ being formed in the body. It is the discrepancy between the $CO_2$ in the expired air during the saturation process and the $CO_2$ being produced in the body, which is the measure of the $CO_2$ absorbed. The $CO_2$ which is being produced in the body at any moment is equivalent to the $O_2$ consumed multiplied by the respiratory quotient. It will be observed that the $O_2$ consumed during the course of the experiment is 2546 cc., and that the respiratory quotient is 0.72. The $CO_2$ produced in the body is, therefore, $0.72 \times 2546 = 1832$ cc. The difference between the $CO_2$ produced in the body and the $CO_2$ which appears in the expired air represents the $CO_2$ which has been absorbed by the body: 1832 cc. $-1462.4$ cc. $= 369.6$ cc.

*The volume of $CO_2$ absorbed by the blood buffers.* The $CO_2$ content of the blood taken while the cat was breathing room air was 40.8 volumes per cent, and the $CO_2$ content of the blood taken at the termination of the experiment, while the cat was still breathing the $CO_2$ mixture, was 62.1 volumes per cent. The difference between these two values (62.1 volumes per cent $-$ 40.8 volumes per cent) is 21.3 volumes per cent and represents the $CO_2$ absorbed as a result of the increased alveolar $CO_2$ tension.

It is found in the experiment cited that the $CO_2$ capacity of the blood taken while the cat is breathing room air is 3.6 volumes per cent higher than the blood taken while the animal is breathing the $CO_2$ mixture. The reasons for this loss of $CO_2$ capacity which occurred in all but one experiment will not be discussed in this paper. In order that full credit may be given to the buffers of the blood, however, this loss is added to the 21.3 volumes per cent of $CO_2$ absorbed, making a total of 24.9 volumes per cent. This is the amount of $CO_2$ which would be taken up by the normal blood as a result of the increased alveolar $CO_2$ tension if it had suffered no loss of base. In order to determine the blood volume we must assume that the blood bears a fixed ratio to the body weight. Erlanger (1921) shows that the blood volume of the cat varies from 4.5 per cent to 6.8 per cent of the body weight. He cites four different methods for blood volume determination. I have taken the average value of 5.5 per cent as representative of the ratio of blood volume to body weight. There is, however, the possibility that the high $CO_2$ content of the blood, resulting from the conditions of the experiment, tends to increase the blood volume at the expense of the tissue fluids. If this should prove to be the case, the ratio of blood volume to body weight would be slightly higher than the above figure. Since the weight of the cat was 3403 grams, the blood volume will be 5.5 per cent of 3403 = 187 cc.

We have seen that, under the conditions of the experiment, the blood was capable of taking up 24.9 volumes per cent of $CO_2$. The total volume of $CO_2$ absorbed will then be equivalent to $0.249 \times 187 = 46.6$ cc.

*The volume of $CO_2$ absorbed by the tissue buffers.* If we deduct the volume of $CO_2$ absorbed by the blood from the volume of $CO_2$ absorbed by the entire body the remainder will be the volume of $CO_2$ taken up by the tissues.

$$369.6 \text{ cc.} - 46.6 \text{ cc.} = 323.0 \text{ cc.}$$

It is then found that the $CO_2$ which resides in the tissues (exclusive of the blood) represents $\frac{323.0}{369.6}$ or 87.4 per cent of the total $CO_2$ absorbed.

*The gradient of the $CO_2$ absorption curve for the blood.* Since the segment of the $CO_2$ absorption curve which we are concerned with consists of a straight line, the curve may be determined by two points. Two samples of blood were equilibrated in tonometers at known $CO_2$ tensions. At a tension of 102.8 mm. of $CO_2$ the blood yielded 69.9 volumes per cent of $CO_2$, and at a tension of 47.9 mm. of $CO_2$ it yielded 49.9 volumes per cent of $CO_2$. Thus a rise of (102.8 − 47.9 =) 54.9 mm. of $CO_2$ tension brought about the absorption of (69.9 − 49.9 =) 20.0 volumes per cent of $CO_2$. The gradient of the absorption curve is, therefore, $\frac{20.0 \text{ volumes per cent}}{54.9 \text{ mm. } CO_2 \text{ tension}} =$ 0.364 volumes per cent for a rise of 1 mm. in $CO_2$ tension.

*The gradient of the $CO_2$ absorption curve for the tissues.* If the volume of $CO_2$ in cubic centimeters be divided by the weight of the tissues in grams, we shall have the volumes per cent of $CO_2$ absorbed by 1 gram of tissue. The tissues absorbed 323 cc. of $CO_2$. The weight of the tissues is equivalent to the body weight minus the weight of the blood: 3403 grams − 187 grams = 3216 grams. The $CO_2$ absorbed by the tissues is, therefore, $\frac{323}{3216}$ = 10.05 volumes per cent.

We have seen that a rise of 1 mm. of $CO_2$ tension causes the blood to take up 0.364 volume per cent of $CO_2$. The absorption of $\frac{24.9}{0.364}$ volumes per cent of $CO_2$ must, therefore, indicate a rise in the alveolar $CO_2$ tension of $\frac{24.9}{0.364}$ = 68.4 mm. If, now, we divide the volumes per cent of $CO_2$

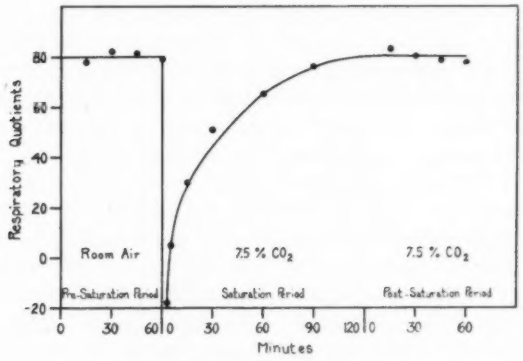


Fig. 2. The saturation process

absorbed by the tissues by the increase in alveolar $CO_2$ tension we shall then find the gradient of the $CO_2$ absorption curve for tissue: $\frac{10.05}{68.4}$ = 0.147 volume per cent $CO_2$ for 1 mm. rise in the alveolar $CO_2$ tension. The validity of such a curve depends upon the assumption that, as is the case with blood, the portion of the curve that we are concerned with resolves itself into a straight line, and can, therefore, be determined by two points.

Discussion. *The saturation process.* The saturation process can best be understood by reference to figure 2. The respiratory quotients are represented against the ordinate and the time in minutes is represented along the abscissa. The points in this figure, which signify the value of the respiratory quotient at different phases of an experiment, are not taken from a single experiment; they are taken from two or more actual experi-

ments, but are entirely representative of the events which take place in every experiment which involves the saturation of the tissues with $CO_2$.

The first four points of figure 2 represent the respiratory quotients taken at 15-minute intervals while the cat is breathing room air. This is the pre-saturation period. These respiratory quotients serve as a basis of comparison for any changes that may occur in the respiratory quotient during the saturation period. The gas mixture is then turned on and the expired air is collected in the collecting spirometer. This is the saturation period. The immediate effect of breathing the $CO_2$ gas mixture is an abrupt fall in the respiratory quotient, caused by the fact that the $CO_2$ tension in the blood is so high that the $CO_2$ that is being formed in the tissues cannot escape into the blood. On the contrary, the negative respiratory quotient shows that the expired air contains less $CO_2$ than the inspired air. This means that the tissues are not only absorbing all the $CO_2$ being produced in them but are also taking up $CO_2$ from the blood. After breathing the $CO_2$ mixture for an hour the respiratory quotient has risen in the instance cited to 0.62, showing that the tissues are still absorbing $CO_2$. It is not necessary to find the respiratory quotient during the saturation period except in so far as it is of interest to determine the nature of the saturation curve. After about two hours the collection of the expired air is discontinued, the volume as indicated on the spirometer scale is recorded and a sample withdrawn for analysis. The respiratory conditions, however, are allowed to continue unaltered for another hour, during which period four more respiratory quotients are taken at 15-minute intervals. This is the post-saturation period. It will be observed that, notwithstanding the fact that the cat is still breathing 7.5 per cent $CO_2$, the respiratory quotients have returned to the normal value for the respiratory quotients of the pre-saturation period taken while the cat was breathing room air.

The return of the respiratory quotient to the normal level signifies that saturation is complete. The animal is functioning perfectly normally, only under the influence of a higher $CO_2$ tension throughout the body. Furthermore, under favorable conditions, the fall in the rate of metabolism due to the rise in the $CO_2$ tension, is insignificant.

Since the calculation of the $CO_2$ which is produced in the body depends upon the respiratory quotient (see experiment 3) it is necessary that the respiratory quotient taken during the post-saturation period should agree very closely with the respiratory quotients taken during the pre-saturation period. In the event that they do not closely approximate the mean value, the experiment must be discarded, since it would be impossible to know which respiratory quotient to take as the determining factor of $CO_2$ production.

*The escape of $CO_2$ within the body.* Before we can with assurance give

credit to the tissue buffers for all of the $CO_2$ which is apparently absorbed by them under the conditions of this experiment, it is necessary to ascertain whether or not some of the $CO_2$ has escaped from the body through some outlet other than the lungs. There are at least two possibilities of escape: first, by the conversion of $CO_2$ into urea which would then be excreted by the kidneys; and secondly, by the diffusion of $CO_2$ through the skin.

The escape of $CO_2$ through the kidneys was tested for by comparing the respiratory quotient of the intact animal with the respiratory quotient of the same animal after the ureters had been tied off. Had there been any escape of $CO_2$ via the kidneys there would have been a rise in the respiratory quotient after ligating the ureters, since the $CO_2$ which had been passing out through the kidneys must now appear in the expired air. I found no such rise, however, the respiratory quotient remaining unaffected by ligation of the ureters.

TABLE 1

*The blood buffers and tissue buffers compared*

| EXPERIMENT | $CO_2$ ABSORPTION GRADIENT OF BLOOD* | $CO_2$ ABSORPTION GRADIENT OF TISSUES* | PER CENT OF TOTAL $CO_2$ HELD BY TISSUES |
|---|---|---|---|
| 1 | 0.458 | 0.311 | 92.2 |
| 2 | 0.320 | 0.101 | 84.3 |
| 3 | 0.364 | 0.147 | 87.4 |
| 4 | 0.293 | 0.134 | 88.7 |
| 5 | 0.276 | 0.100 | 86.1 |
| 6 | 0.277 | 0.158 | 90.7 |
| Average..... | 0.331 | 0.160 | 88.2 |

* The $CO_2$ absorption gradient is the volume per cent of $CO_2$ absorbed by 1 gram of blood or of mixed tissue when the $CO_2$ tension is increased by 1 mm. Hg.

The method by which the diffusion of $CO_2$ through the skin was tested for will be described in a paper to be published later. It was found that $CO_2$ does not diffuse through the skin of the cat. $CO_2$ is given off by the skin but it is balanced by the consumption of $O_2$ in such proportions as fall within the limits of the respiratory quotient normal to the cat. Stated in other words, the skin respires, and the $CO_2$ that is thus given off is in no way dependent upon the $O_2$ of the inspired air.

*The blood buffers and tissue buffers compared.* The experimental results appear in table 1. In column 2 is the gradient of the $CO_2$ absorption curve for 1 gram of blood. In column 3 is the gradient of the $CO_2$ absorption curve for 1 gram of tissue, or the volumes per cent of $CO_2$ which will be absorbed by 1 gram of mixed tissue when the $CO_2$ tension in equilibrium with it has been raised 1 mm. of mercury. In column 4 is the per cent of the total $CO_2$ absorbed by the body which is found to reside in the

tissues, the balance being accounted for by the blood. The average gradient for blood is 0.331 and for tissue 0.160 volume per cent of $CO_2$ absorbed by a rise of 1 mm. $CO_2$ tension. In experiment 5 both the blood and the tissues were far above the average in their capacity to combine with $CO_2$.

The gradients of the $CO_2$ absorption curves for blood and for tissue, the values for which appear in columns 2 and 3 of table 1 are shown graphically in figure 3. It is evident that, although the average capacity of the blood to buffer $CO_2$ is about twice as great as the buffering capacity of the mixed tissues, the maximum capacity of the tissues is greater than the minimum capacity of the blood. There is thus a tendency for the delimiting values of the buffering capacity of blood and tissue to overlap, which is represented by the area between AD and AE. The maximum and minimum variations in the buffering capacity of the tissues is only slightly greater than the variations for the blood.

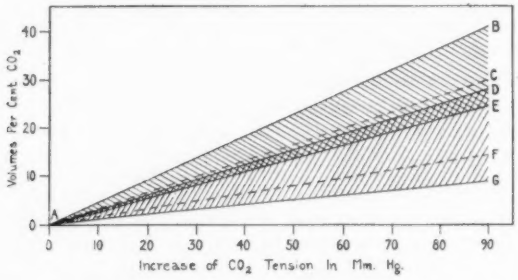


Fig. 3. The $CO_2$ absorption curve for the blood and for the mixed tissues, showing the volumes per cent of $CO_2$ absorbed as the $CO_2$ tension is increased. *AB*, maximum value for blood; *AE*, minimum value for blood; *AC*, average value for blood; *AD*, maximum value for tissues; *AG*, minimum value for tissues: *AF*, average value for tissues.

It was observed that the arterial blood taken at the termination of these experiments was dark, due to inadequate oxygenation. Notwithstanding the fact that the artificial ventilation was considerably in excess of the respiratory volume normal to the animal under experimentation, it appears that the effect of introducing air into the lungs under positive pressure is to dilate the more delicate ramifications of the dead air space and thus to prevent it from reaching the alveoli. For technical reasons it was impossible either to increase the respiratory volume or to increase the $O_2$ in the respired air in order to increase the tension of the alveolar $O_2$.

If lactic acid is being produced in the tissues as a result of oxygen lack, we should expect it to react with the alkali buffers to the exclusion of the $H_2CO_3$. There is the possibility, therefore, that the capacity of the tissues

to buffer acid is somewhat greater than the present series of experiments indicate. Experiments are now in progress involving a modification of the technique, in which the $O_2$ per cent of the respired air is twice as great as in the experiments herein reported. Under these conditions oxygenation of the blood should be complete.

SUMMARY

1. A method is described for determining the total volume of $CO_2$ which the body can absorb as a consequence of a given rise in alveolar $CO_2$ tension.

2. The capacity of the mixed tissues of the body to buffer acid is compared with the buffering capacity of the blood.

3. The per cent of total acid formed in the body which is buffered by the tissues has been determined.

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# ON THE MECHANISM OF PREVENTION OF FATAL PARATHYROID TETANY BY THE ORAL ADMINISTRATION OF MAGNESIUM CHLORIDE

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There are in existence at present numerous methods for the prevention and relief of tetany following parathyroidectomy, chief of which are: intravenous injection of large quantities of Ringer's solution (1), oral administration of calcium lactate (2), controlled diet of milk, bread and lactose (3), oral administration of strontium lactate (4), subcutaneous injections of parathyroid extracts (5), and oral administration of large quantities of magnesium chloride (6), which work was recently confirmed by Wenner, using magnesium lactate (7). The oral administration of ammonium chloride (8) to control parathyroid tetany has been attended with somewhat less success than the other methods enumerated.

Luckhardt and his associates, using magnesium chloride orally, attributed their results as due primarily to depression of the central nervous system, for most of their animals showed pronounced symptoms such as are seen when either magnesium sulfate or magnesium chloride is injected subcutaneously. They also pointed out that magnesium chloride given orally did not act prophylactically by leading to mobilization of blood calcium, for the blood calcium level in most cases dropped very low (4 to 5 mgm. per 100 cc. of blood serum). They concluded, therefore, that the neuro-muscular phenomena, so characteristic of parathyroid tetany, were prevented by the oral administration of magnesium chloride because of the depression of the central nervous system brought about by the presence of magnesium ions.

In view of this work, it seemed feasible to effect the same result by means of other drugs which are known to have a marked depressing action upon the central nervous system. Several such drugs were considered, but the work to be reported was done with morphine sulfate.

LITERATURE. As early as 1892, Gley (9) reported an attempt to preserve life in completely thyroidectomized dogs by the oral administration of potassium bromide. He reported on but two dogs, and these lived so short a time after operation that it seems doubtful if the potassium bromide had any beneficial effect. In an attempt to check up on this work,

potassium bromide was administered both intravenously in dogs in tetany and orally in normal dogs. It was found that large amounts of potassium bromide (2 to 4 grams) given rapidly by the intravenous route caused death, and that the same dose when injected repeatedly, but at a much slower rate, was not fatal, but, at the same time, had no beneficial effects upon the tetanic symptoms. Small doses (4 grams per day) given orally produced no signs of depression in normal dogs, while large doses (12 to 18 grams per day) caused severe gastro-intestinal disturbances which resulted in prolonged attacks of retching and vomiting, leaving the animal in a condition of exhaustion.

Sodium barbital was found undesirable because of its prolonged action and pronounced medullary depression, which, added to the post-tetanic depression present in parathyroidectomized animals after an attack, does not bode well for recovery. There is also the factor of marked variations in the susceptibility of different dogs to this drug which makes it necessary to work out the dosage for each animal individually. A drug such as paraldehyde, however, might be more desirable because of its rapid action and rapid excretion by the lungs which prevents any unduly prolonged depression. With these facts in mind, it was therefore decided to use morphine sulfate, because dogs are very tolerant to it and it acts quickly but not over too great a period of time.

EXPERIMENTAL METHODS. Dogs were used exclusively. In many cases, the susceptibility of the animals was determined for a given dose of morphine sulfate. For several days prior to operation the animals were put on a meat diet consisting of one-half pound cooked hamburger daily. Blood samples were drawn for normal calcium determinations, the method used being Tweedy's (10) modification of the Kramer-Tisdall method. The animals were completely thyroparathyroidectomized, after which blood samples were again taken at various times for calcium determinations. When the animals went into tetany they were given morphine sulfate subcutaneously, as needed. Whenever possible, blood calcium determinations were made at the height of tetany, while free from tetany but under the effects of morphine sulfate, and again, when completely out from under the influence of morphine and free from tetany. If tetany supervened spontaneously or as a result of the animal eating its tetany-inducing ration of meat, morphine sulfate was again given subcutaneously.

RESULTS. This report is based on six dogs. In five dogs blood calcium determinations were made throughout several experiments. We submit two protocols of typical experiments indicating how morphine sulfate injected as needed over a period of several days cut short the otherwise fatal neuro-muscular attacks of tetany.

*Dog 4. Male. Weight 17.1 kgm.*

| DATE | TIME | BLOOD CA. MGM./ 100 CC. SERUM | MEDICATION | REMARKS |
|---|---|---|---|---|
| *1926* | | | | |
| Feb. 17 | | 9.45 | | Animal O. K. Fed ½ lb. cooked hamburger |
| 18 | | 9.00 | | Animal O. K. Fed ½ lb. cooked hamburger |
| 19 | | 9.32 | | Animal O. K. Fed ½ lb. cooked hamburger |
| 20 | 12:00 m. | 9.12 | | |
| | 1:30 p.m. | | | Thyroparathyroidectomy. |
| | 9:30 p.m. | | | Ate ½ lb. cooked meat with good appetite |
| 21 | 11:00 a.m. | | | Fed. ½ lb. cooked meat |
| | 12:00 m. | 7.90 | | Nictitating membrane showing |
| | 9:00 p.m. | | | Very active. No signs of tetany |
| 22 | 11:45 a.m. | | | Fed 1 lb. cooked meat |
| | 3:00 p.m. | 6.77 | | Animal in tetany, Twitchings in all four legs. Body hot to touch. Slight hyperpnea |
| | 3:10 p.m. | | ⅜ gr. morph. sulf. | Vomited meat. Hyperpnea increased |
| | 4:45 p.m. | | | Animal sleeping quietly. Only barely perceptible shoulder-twitching |
| | 5:30 p.m. | | | Sleeping. Complete absence of tetany. Legs relax. Respiration 14 per minute |
| | 8:55 p.m. | | | Dog awake and active. Shows no signs of tetany. Eyes and nose normal |
| 23 | 2:30 p.m. | 6.54 | | Still active. No signs of tetany. Fed ½ lb. cooked meat |
| | 7:15 p.m. | 6.13 | ⅜ gr. morph. sulf. | Shows tetany in legs and diaphragm. Body warm. |
| | 7:30 p.m. | | | Standing and exhibiting hyperpnea |
| 24 | 7:50 a.m. | 6.45 | ½ gr. morph. sulf. | Generalized tetany. Became stiff-legged and fell on side several times. Hyperpnea |
| | 8:25 a.m. | | ½ gr. morph. sulf. | |
| 24 | 11:00 a.m. | | ⅛ gr. morph. sulf. | Hyperpnea (114 per min.) only evidence of tetany. Otherwise relaxed |
| | 12:30 p.m. | 6.48 | | Completely relaxed and quiet |
| | | | | Resp. 26. Given 400 cc. $H_2O$ by stomach tube |

*Dog 4. Male. Weight 17.1 kgm.—Continued*

| DATE | TIME | BLOOD CA. MGM./ 100 CC. SERUM | MEDICATION | REMARKS |
|---|---|---|---|---|
| *1926* | | | | |
| | 8:30 p.m. | | | Animal has been in good condition all afternoon. No signs of tetany |
| 25 | 11:30 a.m. | | | Condition O.K. Fed ½ lb. cooked meat |
| | 11:00 p.m. | | | Curled up asleep. Has shown no symptoms of tetany all day |
| 26 | 8:00 a.m. | 4.76 | 1 gr. morph. sulf. | Found in state of moderately violent tetany. Symptoms generalized |
| | 8:20 a.m. | | | Hyperpnea setting in. Leg jerkings more marked |
| | 10:30 a.m. | | | Lying perfectly relaxed. No signs of tetany |
| | 2:00 p.m. | | | 350 cc. $H_2O$ stomach tube |
| | 3:15 p.m. | | | Ate ½ lb. cooked meat. Condition O. K. |
| | 10:10 p.m. | | 1 gr. morph. sulf. | Found in advanced state of tetany. Hyperpnea—bloody froth—enophthalmos |
| | 10:55 p.m. | 4.32 | 1½ gr. morph. sulf. | Condition improved slightly |
| | 11:45 p.m. | | | Attack subsiding. Legs stiff but show no jerkings. Temperature 108.0 |
| 27 | 8:00 a.m. | | | Still under influence of morphine. Given 200 cc. $H_2O$ by stomach tube |
| | 1:30 p.m. | | 10 gm. Ca. lactate and 10 gm. $CaCO_3$ by stomach tube | Animal depressed but shows no signs of tetany |
| *Mar. 22 | 10:00 a.m. | | | Animal O. K. Fed ½ lb. cooked meat |
| | 10:20 p.m. | 7.79 | | No signs of tetany except for distended leg vein |
| 23 | 1:00 p.m. | | | Fed ½ lb. cooked meat. No signs of tetany all day |

*From February 28 to March 21 the animal was kept on a fattening diet of meat, bread, milk, bones and a daily dose of 20 gm. calcium. On March 22, he was put back on a meat diet with no calcium.

*Dog 4. Male. Weight 17.1 kgm.—Continued*

| DATE | TIME | BLOOD CA. MGM./ 100 CC. SERUM | MEDICATION | REMARKS |
|---|---|---|---|---|
| *1926* | | | | |
| Mar. 24 | 4:15 p.m. | | | Fed ⅓ lb. cooked meat. No signs of tetany all day |
| 25 | 5:30 p.m. | | | Fed ½ lb. meat. Animal appears perfectly normal |
| 26 | 7:45 p.m. | | | Fed 1 lb. cooked meat. Animal has been normal all day |
| 27 | 10:00 a.m. | 6.79 | ¾ gr. morph. sulf. | Found in advanced stage of tetany. Bloody froth at mouth, hyperpnea, etc. |
| | 11:45 a.m. | | | Still has moderate hyperpnea. Lying down quietly with only occasional slight fibrillations |
| | 6:00 p.m. | | | Animal appears normal again. Ate ⅔ lb. cooked meat |
| | 11:00 p.m. | | | Animal very active. No signs of tetany |
| 28 | 11:30 a.m. | | | Active and normal. Ate ½ lb. cooked meat |
| | 1:30 p.m. | | 1 gr. morph. sulf. | M. S. given as prophylactic dose |
| 29 | 11:00 a.m. | 8.52 | | Leg fibrillations while drawing blood. Otherwise animal active and normal all day. Fed ½ lb. of cooked meat |
| 30 | 2:00 p.m. | | | Animal O.K. Fed 1½ lb. fresh raw meat |
| | 8:45 p.m. | 7.06 | 1 gr. morph. sulf. | Found in advanced stage of tetany. Clonic and tetanic seizures, bloody froth at mouth and hyperpnea. Morphine caused vomiting |
| | 9:15 p.m. | | | Twitchings, slightly less marked. Resp. 198 per min. Temp. 106.2 |
| | 9:50 p.m. | | 1 gr. morph. sulf. | Not much improvement |
| | 10:10 p.m. | | | Resp. 194. Temp. 105.8 |
| | 10:45 p.m. | | | Resp. 140. Tetany diminishing |
| | 11:00 p.m. | | ½ gr. morph. sulf. | |
| Apr. 1 | 8:45 a.m. | | | Animal O. K. and active. Weakness in hind limbs. No signs of tetany |
| | 3:00 p.m. | 8.16 | | Animal O. K. |
| | 9:30 p.m. | | | Active. No signs of tetany |
| 2 | | | | Animal O. K. all day |
| 3 | 11:00 a.m. | | | Fed ½ lb. cooked meat. O. K. all day |

*Dog 4. Male. Weight 17.1 kgm.—Concluded*

| DATE | TIME | BLOOD CA. MGM./ 100 CC. SERUM | MEDICATION | REMARKS |
|---|---|---|---|---|
| *1926* | | | | |
| Apr. 4 | 11:00 a.m. | | ¾ gr. morph. sulf. | Found in beginning tetany |
| | 4:30 p.m. | | | Slight fibrillations |
| | 11:00 p.m. | | | Animal O. K. and active |
| 5 | 2:00 p.m. | | | Fed ½ lb. cooked meat. Condition O.K. |
| | 9:30 p.m. | 7.06 | 1 gr. morph. sulf. | Vomited small amount of meat which he then ate. Shows signs of impending tetany—uneasy—salivation—fibrillations of fore and hind legs when placed on back. Skin hyperemic |
| 6 | 3:30 p.m. | | | Given ½ lb. cooked meat. Condition O. K. |
| | 4:30 p.m. | | | Vomited some meat |
| | 8:30 p.m. | 6.16 | 1¼ gr. morph. sulf. | Found in advanced state of tetany. Generalized twitchings and jerkings—hyperpnea. jaw snapping, etc. |
| | 8:50 p.m. | | | Respiration 200 per min. |
| | 9:25 p.m. | | | Respiration 130. Animal lying on side breathing noiselessly. Only slight fibrillations in thighs, the fore limbs being completely relaxed |
| | 9:50 p.m. | | | Dog got up and walked around showing no signs of tetany. Weakness in hind limbs due to morphine |
| 7 | 10.00 a.m. | | | No signs of tetany. Animal active |
| | 7:30 p.m. | 6.12 | | Found in advanced state of violent tetany |
| | 7:45 p.m. | | 1¼ gr. morph. sulf. | |
| | 8:15 p.m. | | 1¼ gr. morph. sulf. | Symptoms still very marked |
| 8 | 12:30 a.m. | | | Gross symptoms gone—rapid quiet breathing—occasional jerking in hind legs. Body much cooler |
| | 9:45 a.m. | | | Occasional jerkings and twitchings. Has extremely nervous appearance of a "dope." Body and nose cool |
| | 11:15 a.m. | 6.11 | | |
| | 11:30 a.m. | | 10 gm. Ca. lactate and 10 gr. $CaCO_3$ by stomach tube | |

From April 9 to April 19, the animal was kept on a fattening diet of bread, milk, bones, meat, and 20 gm. ca. daily. Between April 20 and May 15, he was kept on a diet of raw or putrid meat (no Ca.) without inducing tetany.

*Dog 1. Male. Weight 13.1 kgm.* Normal blood clacium value averaged 9.31 mgm. per 100 cc. serum. On December 5, 1925, the animal was then thyroparathyroidectomized and kept on morphine medication for four days, during which time five attacks of tetany were successfully controlled by morphine. The blood calcium dropped as low as 4.5 to 5 mgm. per 100 cc. serum. He was then put on calcium for 29 days, after which it was necessary to use croton oil and putrid meat to induce tetany. This attack was also successfully controlled by morphine. The animal is still alive (May 19, 1926) and is in good condition.

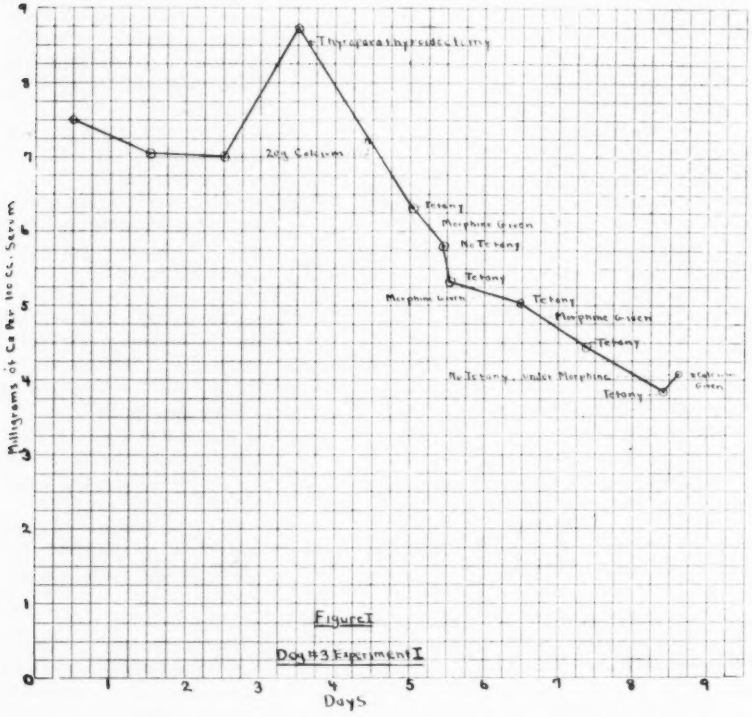


Fig. 1. Graph showing changes in blood calcium level in a thyroparathyroidectomized dog while the tetany was being controlled with morphine sulfate.

*Dog 2. Male. Weight 16.8 kgm.* Normal blood calcium averaged 10.18 mgm. /100 cc. serum. January 31, 1926, the animal was completely thyroparathyroidectomized. The animal was kept on morphine medication for eight days during which period he showed definite symptoms of tetany but three times, and these were promptly suppressed by morphine. The blood calcium, however, dropped as low as 4.5 mgm./100 cc. serum. The dog was then put on a calcium diet but developed a condition of cachexia and was therefore sacrificed.

*Dog 3. Male. Weight 10 kgm.* Normal blood calcium averaged 7.58 mgm./ 100 cc. serum. February 13, 1926, complete thyroparathyroidectomy. Kept for

five days without calcium. Showed five attacks of tetany all controlled by morphine. Blood calcium dropped as low as 3.80 mgm./100 cc. serum. Dog then put on calcium medication for eight days and then taken off. Kept off calcium for seven days, during which time there were four attacks of marked tetany all adequately controlled with morphine. Blood calcium as low as 4.00 mgm./100 cc. serum. Put back on calcium for five days more. Taken off calcium diet but did not show any marked symptoms until the sixth day at 6:00 p.m., when he seemed to be much disturbed. He was given neither morphine nor calcium, and when seen again at 9:30 p.m. was found dead. Autopsy showed distended lungs filled with air. The animal had apparently died from laryngeal spasm as a result of tetany.

*Dog 5. Female. Weight 19.5 kgm.* Normal blood calcium 8.30 mgm./100 cc. March 24, 1926, complete thyroparathyroidectomy. Had four attacks of marked tetany in six days all successfully treated with morphine. The blood calcium dropped as low as 5.28 mgm./100 cc. serum. On the morning of the seventh day at 7:00 a.m. the animal was seen to be in a depressed condition, but was given no morphine. At 8:30 a.m. when seen again, was dead. The legs were limp but the jaws were tightly set. Had apparentiy died in tetany.

*Dog 6. Male. Weight 7.5 kgm.* Thyroparathyroidectomized on April 15, 1926. Blood calciums were not determined on this animal. This animal was brought out of four successive attacks of tetany on four successive days by use of morphine sulfate. The animal is still alive on calcium medication.

*Tabular review of results showing calcium levels during tetany and number of attacks treated with morphine sulfate*

| DOG | MEDICATION | DAYS | TETANY ATTACKS | NORMAL BLOOD CALCIUM | TETANY LEVEL CALCIUM | REMARKS |
|---|---|---|---|---|---|---|
| 1 | Morphine $SO_4$ | 4 | 5 | 9.31 | 4.5 | |
| | Calcium | 29 | 0 | | | |
| | Morphine | 10 | 1 | | | This attack induced by feeding putrid meat and croton oil. Dog still alive and in good condition (5/19/26) |
| 2 | Morphine | 8 | 3 | 10.18 | 4.5 | |
| | Calcium | 6 | 0 | | | Animal developed a cachectic condition and was sacrificed |
| 3 | Morphine | 5 | 5 | 7.58 | 3.80 | |
| | Calcium | 8 | 0 | | | |
| | Morphine | 7 | 4 | 7.58 | 4.00 | |
| | Calcium | 5 | 0 | | | |
| | | 6 | | | | Appeared disturbed at 6:00 p.m. of 6th day. No medication. 9:30 p.m. found dead. Lungs distended and filled with air. Apparently died in tetany |
| 4 | Morphine | 7 | 5 | 9.22 | 4.32 | |
| | Calcium | 21 | 0 | | | |
| | Morphine | 18 | 6 | 9.22 | 6.20 | |
| | Calcium | 8 | 0 | | | After the 56th day after operation the animal did not go into tetany when given no medication |

*Tabular review of results showing calcium levels during tetany and number of attacks treated with morphine sulfate—continued*

| DOG | MEDICATION | DAYS | TETANY ATTACKS | NORMAL BLOOD CALCIUM | TETANY LEVEL CALCIUM | REMARKS |
|---|---|---|---|---|---|---|
| 5 | Morphine | 6 | 4 | 8.30 | 5.28 | Seemed depressed 7:00 a.m. of 7th day. No morphine given. Found dead 8:30 a.m. with jaws set. Apparently died in tetany |
| 6 | Morphine | 4 | 4 | | | This animal was then allowed to go into tetany several times at intervals between calcium medication and was brought out with morphine. No blood calciums were determined |

Discussion. From the protocol of dog 4 it is seen that the phenomenon of parathyroid tetany is cyclic in character, and that the animal free from tetany and free from the effects of morphine has a blood calcium level at least as low as that of the animal in tetany. This holds for the animal when newly parathyroidectomized, but in the second series of experiments, started thirty days after the operation, we find the animal in violent tetany with a blood calcium of 7, and out of tetany with blood calcium level above 8 mgm./100 cc. serum. That is, the blood calcium level does not always seem to parallel the condition of the animal when there is no calcium intake. This at once raises the old question as to whether the blood calcium levels are the cause or effect of tetany or whether they play no actual rôle in the onset of the neuromuscular phenomena. The fact that a newly operated animal, after being carried through its first attack of tetany by means of morphine sulfate, shows a blood calcium level low enough to be a tetany level, seems to indicate that the attack of tetany was a result of a more complex physiological derangement than a mere lowering of the blood calcium content. Otherwise we should expect the animal to immediately relapse into a state of tetany as soon as the effects of the morphine wear off, but that is not the case.

The pharmacological properties of morphine aid in explaining its rôle in the suppression of tetany by reducing the condition of alkalosis. Morphine, in doses ($\frac{3}{8}$–$2\frac{1}{2}$ grains) as given to the dogs used, has a marked depressing action upon the respiratory center and has been observed to reduce a respiratory rate of 220 per minute down to as low as 9 to 12 per minute within one to one and one-half hours. With so slow a respiration, hyperpnea alkalosis is prevented or abolished.

Similarly, a cold bath given a dog in tetany has the property of reducing the symptoms by reducing the hyperpnea alkalosis, but its mode of action

is different from that of morphine. As dogs have no sweat glands, they lose body heat by panting, which evaporates water from the saliva in the mouth and water from the lungs. Dogs in tetany usually exhibit an hyperpyrexia, which necessitates panting to reduce the body temperature, and hyperpnea even in normal dogs and man produces an alkalosis due to overventilation. Such an alkalosis increases the excitability of the C.N.S. and may and does induce seizures of tetany. If, then, a dog has tetany due to excision of the parathyroid glands, and in a tetany attack pants, he probably superimposes the tetany of hyperpnea upon the parathyroid tetany and makes his condition worse. Then, giving a cold water bath reduces his body temperature, obviates the necessity of panting, stops the tetany due to hyperpnea, and improves his general condition.

On several occasions the animals were found in the last stages of tetany, with the respiratory center failing. When given morphine subcutaneously, the failing respiratory center was stimulated to renewed activity, and, as the second stage of morphine depression came on, the respiration gradually dropped, thereby reducing the alkalosis and suppressing the tetany. This double action of morphine as a primary stimulant and a secondary depressant of the respiratory center marks it as a very valuable drug which can be used in cases where the respiratory center is failing and the animal is too far gone to use calcium, which requires a much longer period of time to act. It is also not an uncommon occurrence for animals in advanced tetany to have spasms of the pyloric and cardiac sphincters, which prevent calcium from passing into the small intestine for absorption. In such cases morphine can be used to preserve the life of the animal. In short, morphine gives promise of being a very useful drug for the control of parathyroid tetany when other measures are either inadequate, not at hand, or before it is safe to employ other means. It is well known that the passage of a stomach tube at the height of tetany may induce a fatal attack, but with the animal under morphine sulfate this does not happen and the stomach tube may be readily passed to administer calcium salts. When the dog recovers from the morphine, he is well under the control of the administered calcium salt.

The continued use of morphine over a number of days, in the control of tetany, leads to certain gastro-intestinal disturbances which would naturally contra-indicate its use as the sole therapeutic agent in carrying dogs through their tetany period (40 to 60 days). As a rule, it invokes in parathyroidectomized animals an abhorrence for food and water, it being necessary to give water by stomach tube. There is also an acute constipation and retention of urine. When the animal does defecate, the stools are often very bloody and of a semi-liquid consistency. The dogs that came to autopsy when in this condition did not, however, show any

gross pathology in either the small or large intestine. Tatum (11) observed while working with dogs on chronic morphinism that after the administration of morphine sulfate over a period of several days, there appeared an increased excitability in the animals. This did not seem to increase noticeably the tetany seen subsequent to thyroparathyroidectomy, although after being put back on calcium medication some of the dogs appeared to be subject to hallucinations.

## SUMMARY

1. Completely thyroparathyroidectomized dogs can be successfully carried through 4 to 5 successive attacks of tetany occurring over periods of from 4 to 7 days by means of morphine sulfate which is a known depressant of the central nervous system.

2. The action of morphine sulfate in this respect is similar to that of magnesium chloride given orally to prevent the occurrence of tetany after complete thyroparathyroidectomy.

3. When morphine is used to control parathyroid tetany the blood calcium levels do not always seem to parallel the condition of the animal; i.e., the blood calcium level during one attack of tetany may be higher than that of a previous tetany-free period.

4. Morphine gives promise of being a valuable emergency agent for the control of parathyroid tetany either before other more slowly acting therapeutic agents take effect or at a time when other methods should not be tried because of the danger of inducing a fatal attack of tetany.

I am greatly indebted to Doctor Luckhardt for his many valuable suggestions concerning the experimental method followed and to Doctor Carlson for his aid in preparing the manuscript for publication. I also wish to thank Mr. S. L. Stern and Mr. Joseph Rozen for their skilful assistance in the surgical work.

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# PERIODIC VARIATIONS IN BLOOD PRESSURE PULSE AND THE PHYSICAL EFFICIENCY TEST[1]

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In connection with investigations of conditions which influence the circulatory efficiency test in its general application, it was considered of interest to study the effect upon it of the monthly cycles of women. This study, carried on over a considerable period of time and under carefully guarded conditions, has yielded data of value from the point of view of monthly rhythm in cardiovascular activities.

The circulatory efficiency test referred to is a test devised by Lt. Col. E. C. Schneider at the School of Aviation Medicine, Mitchel Field. It is in general use in the Air Service of both Army and Navy as well as in many schools and colleges. It gives an estimate of the general physical condition of the subject. It has been found that various factors influence the results of the index and in view of the findings of Moore and Cooper and others regarding a monthly rhythm in women, it was thought possible that the rhythm might be sufficient to influence the use of the index with women. The original paper describing this test appeared in Journal of the American Medical Association (Schneider, 1920). Results of a further study regarding disturbing factors appeared in the Military Surgeon (Schneider, 1923) and the daily variations were also considered (Schneider and Truesdell, 1923).

Each test included pulse and blood pressure after the subject had been reclining five minutes, pulse and blood pressure after standing two minutes, pulse immediately after a standard exercise (stepping on a chair five times in fifteen seconds), time of return of pulse to former standing rate. These factors were then summed up by the table for rating of the circulatory efficiency test. A Tycos sphygmomanometer was used for taking the blood pressure.

The subjects used were six young women all between 20 and 30 years of age, employed in the School of Aviation Medicine as technical or clerical workers. The tests were made at least two hours after a meal and at exactly the same time each day. Careful notes were taken as to any

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abnormal conditions and any tests showing the effects of such conditions were eliminated from the averages. All the young women were in the habit of being used as subjects in various phases of physiological research so the psychic factor was practically nil.

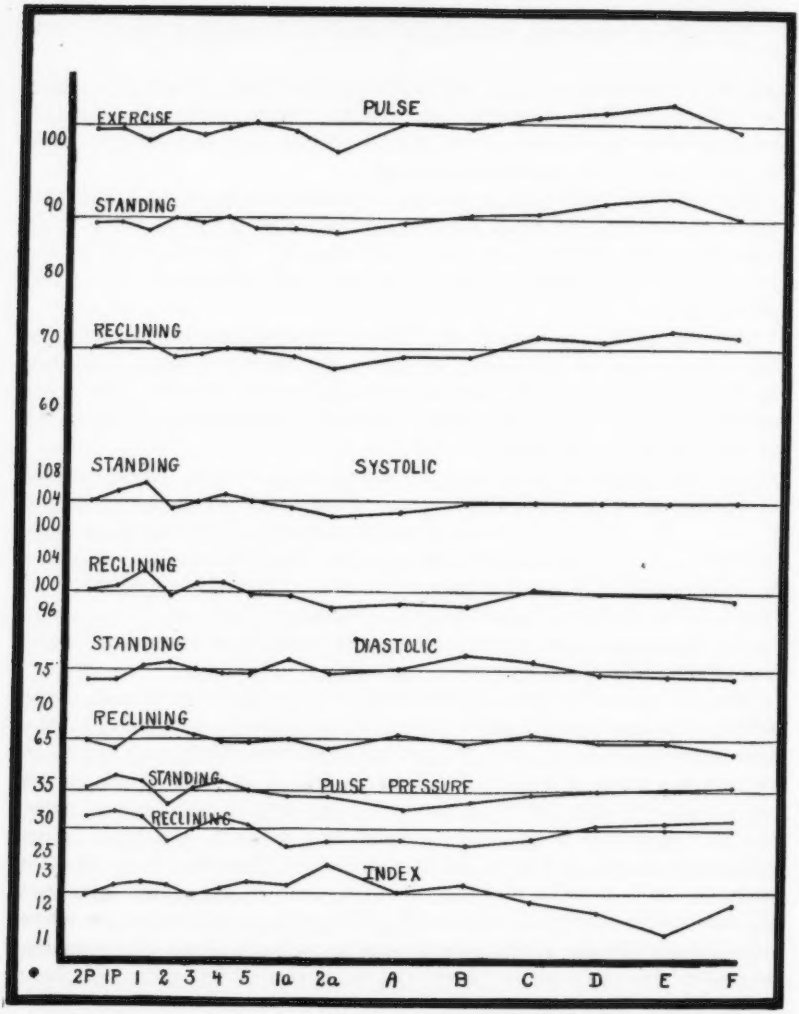
The test was made on the two days previous to the period, on each day of the period, the two days following the period and on alternate days between periods except when Sunday intervened. The number of cycles followed through completely on each subject were: C-11, D-9, G-6, H-8, S-9, T-10.

The data of each factor for each subject were tabulated separately by days, the tests of the mid-period being grouped into six divisions consisting of three or four days each, varying with the length of cycles. The idea of this grouping was to standardize the cycles so that a composite could be made of the whole group.

For each person an individual curve was established by averages of all the cycles on that person, an average being made for each day of the period, for the two days preceding and the two days following the period, and for each of the six inter-period groups. These six individual curves were then averaged to form a composite curve. The extreme variation of single cycles was from 25 to 42 days. Periods varied from 5 to 8 days, but in the composite curve only 5 days are shown because unless all cases were represented the results were misleading.

We gave careful attention to the periodicity study of Moore and Cooper (1923) who have considerable data of blood pressure and pulse in the sitting position and recalculated our averages from the actual figures to percentages to compare our data with theirs. Also because the curves published by Moore and Cooper were for one subject only, we computed the averages from their table of 8 different subjects and have drawn composite curves of seated pulse, systolic and diastolic blood pressure grouping the intermenstrual days to correspond to our grouping so that the two studies might be comparable. We prefer, however, to use curves established from the actual figures because we have found that reducing to percentages gives undue prominence to cases with low pulse and blood pressure and distorts the curve.

The curves of subject 12 presented by Moore and Cooper in their article show four distinct waves in systolic and diastolic pressure, the upper peaks being from 1 to 2 per cent above average and occurring on the fifth, twelfth, twenty-first and twenty-eighth days of a thirty day cycle, this being a composite curve of seven cycles on the same subject. The pulse curve shows two low points on the fourth and twenty-second days and two high points on the seventeenth and twenty-ninth. When, however, we compute from their tables averages of the composite curves of eight different subjects, the form of the curve is different. The systolic is


PULSE
EXERCISE
100
90
STANDING
80
70
RECLINING
60
108
STANDING
SYSTOLIC
104
100
104
RECLINING
100
96
STANDING
DIASTOLIC
75
70
RECLINING
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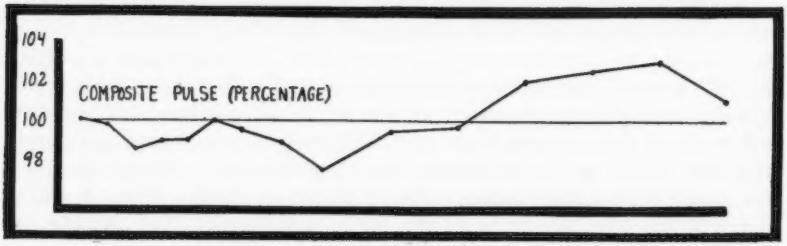
104
102
COMPOSITE PULSE (PERCENTAGE)
100
98

high in the early menstrual period, average for about a week, subaverage for a week, average again for another week and rises for about six days previous to the period. Diastolic follows much the same course except that the rise during the period is specially marked and that there is a rise midway in the intermenstrual period. Pulse has only one big wave, the crest of which is in the middle of the intermenstrual period, 3 per cent above average, the low point being on the first day of the period and 2 per cent below average.

Neither in our curves nor in those which we computed from Moore and Cooper's table have we smoothed the curves by averaging each day with the adjacent days, fearing to lose some possibly significant changes, especially at the beginning of the period.

*Reclining pulse.* The composite curve of the reclining pulse (fig. 1) is slightly above average during the two days preceding and slightly below average during the period. The first half of the intermenstrual period is below average, the latter half above, tending downward again before the period. The six individual curves vary considerably from each other during the period but during the intermenstrual period each follows the composite with striking regularity.

*Standing pulse.* The composite curve of the standing pulse shows a somewhat slower pulse just preceding the period, a hovering near the average during the period, due to the variations of the individual curves, and an intermenstrual curve similar to the reclining pulse, low during the first and high during the latter portion of the curve, tending down again before the period.

*Exercise pulse.* The same curve is found for the exercise pulse except that the period itself is below average, the individual curves being more similar in this respect. The end of the intermenstrual curve falls off more abruptly before the period.

When revised to a percentage basis, the curves, though exaggerated, show the same general course and the seated pulse of Moore and Cooper is somewhat the same except that the menstrual portion of the curve is lower than ours and the first of the intermenstrual is just average where ours is in a subaverage phase. Their peak comes earlier in the intermenstrual period than ours. However, a composite of all the percentage pulse curves (fig. 1) reclining, seated, standing and after exercise gives a curve fairly typical of all. So that in general the pulse is below average during, and above average between periods in a range of 5 per cent.

*Reclining systolic.* The composite curve shows a premenstrual and menstrual period above average, the intermenstrual curve being in the form of a wave the crest of which, only slightly above average, occurs about midway between periods, both before and after this peak the pressure is below average. The individual curves follow the composite quite closely.

*Standing systolic.* The composite standing systolic has a curve almost identical with that of the reclining systolic only 4.5 mm. higher. The pressure is uniformly above average just preceding and in all but one case the curve is above on the first day with a fall to below average at some time during the period in every case. The mid-period varies with the individual, only one of them being as smooth as the composite. The fact that the peaks occur at different points in different individuals makes them neutralize each other in the composite curve, yet each shows a wave tendency, five showing one peak and one showing two peaks. In every case but one the pressure falls below average just before the premenstrual rise.

When these curves are reduced to percentages and compared with the composite seated systolic from Moore and Cooper's data, they do not agree as well as the pulse curves did. In the seated curve the premenstrual and early menstrual curve is high, falling to slightly below normal at the close of the period and reaching the lowest point at the middle of the intermenstrual period, the time when our reclining and standing systolics are at the crest of the intermenstrual wave. The rise just before and on the first day agrees with our cases but the fall which in our cases immediately precedes this rise is lacking. In general the systolic is above average during and below average between periods, the average variation being no more than 5 mm. The changes are not as definite or uniform as those of the pulse, with the exception of a clear cut fall during the latter part of the intermenstrual period and a rise above average during the two days previous to and the first day of the period.

*Reclining and standing diastolic.* The curves of reclining and standing diastolic blood pressure are sufficiently similar to be treated together. Below normal just before the period, they are above normal during the later menstrual period. There is another peak midway between the periods after which there is a fall until the following period. The curve of the seated diastolic from Moore and Cooper's cases follows ours except that their lowest point is before the intermenstrual peak whereas ours is after the peak before the menstrual rise.

*Pulse pressure.* The average curve for pulse pressure shows a fairly distinct swing, reclining and standing being almost identical in form, though the average for reclining is 34.1 mm. and the average for standing 28.5 mm. except for a very marked drop in the second day of the period, the pulse pressure is above average from the intermenstrual division D through the menstrual period. From this point through division C the pulse pressure is subaverage. The lowest point is in this phase, the highest is on the first day previous to the period, the difference between the highest and the lowest being 4.7 mm. in the reclining and 5.4 mm. in the standing.

*The circulatory efficiency rating* of these subjects, a rating with a possible range from 18 as a maximum into minus quantities, based on six factors, namely, *1*, reclining pulse; *2*, standing pulse; *3*, pulse increase on standing; *4*, pulse increase over the standing rate after a standard exercise; *5*, time of return to normal after this exercise; *6*, systolic change from reclining to standing, was also determined and curves established.

Since five of the six factors have to do with pulse rate reactions, the curve of this rating tends to follow inversely the pulse curve, being higher during and just following the period and low between the periods. The total variation shown on the composite curve is from 11 at group E of the intermenstrual period to 13 on the second day after the period, a variation which is very small, the curve being at every point within the range of the average subject and the general average, 12.35, being appreciably above the mean index, 11.3, of 155 regular and reserve fliers examined at Mitchel Field during July, 1923. The individual average index curves have an average swing of 3.2 points, the lowest being 2.2, the highest being 4.2. It seems, then, that the variations are not sufficiently extensive to distort unduly the findings of the index.

*Individual variations.* It was found that individuals differed more or less from the composite curve, but it seemed in nearly every case that these differences were during rather than between the periods.

Case D showed, over a series of nine months, a consistent irregularity on the second day of the period, pulse higher, blood pressure lower and index dropping 1.4 points from the preceding day. Nothing seems to account satisfactorily for this. The subject usually suffered considerable discomfort the first two days, the condition being improved the second day. Subject H defied the general trend of pulses by having a more rapid pulse during the first two days of the period where the majority of the cases slowed. Her symptoms were not abnormal, the only difference being that the duration was eight days. Case G showed a pulse slightly higher than the average during the first two days, her drop coming during the latter part of the period.

SUMMARY. A study of the circulatory efficiency test and the separate factors upon a group of six women during an average of nine monthly cycles discloses:

1. In pulse rate a slower rate during the menstrual and a higher rate in the intermenstrual periods.

2. In systolic pressure a rise previous to and in the early part of the period.

3. In diastolic pressure a rise during the period and another rise midway between periods.

4. In pulse pressure a rise above average during the later intermenstrual and through the menstrual period.

5. In the circulatory efficiency test a somewhat higher rating during the period and a lower rating between periods, the total average swing being from 11 to 13, which is very small.

## CONCLUSION

That both blood pressure and pulse show a monthly rhythm, that these rhythms vary somewhat with individuals but that certain definite trends are discernible. Also that the range of these rhythms is small and seems insufficient to distort unduly the findings of the circulatory efficiency test.

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# THE METABOLIC GRADIENT OF NON-RHYTHMIC STRIPS FROM DIFFERENT LEVELS OF THE TURTLE HEART

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It has long been maintained that certain properties of cardiac tissue differ quantitatively, or follow a changing potential from one region of the heart to another. One of these properties is that of rhythmicity, as pointed out by Gaskell (1883). Other functions also follow the regional gradient, as shown by T. Lewis (1921); and confirmed and extended for segments of the turtle heart by Sollmann and Chu (1925). It appears interesting to determine whether this regional sequence is reflected also in the basal metabolism, i.e., in the rate of $CO_2$ production, analogous to the "metabolic gradient" that Alvarez (1922) demonstrated for the gastro-intestinal tract. This may be done on strips of muscle from different levels of the heart, which must be rendered non-rhythmic to avoid the variable "work-metabolism."

*The apparatus.* The apparatus used for the $CO_2$ determinations in this work is essentially that described by Osterhout (1918) and employed later by several of his co-workers. Some slight modifications were introduced (see appendix A). The apparatus is a closed system in which the contained gases are made to circulate continuously, and in such manner that the $CO_2$ given off by the respiring tissue is led through a M/10,000 $NaHCO_3$ solution appropriately colored with phenolsulphonephthalein. The rate at which $CO_2$ is produced is found by the time required to change the pH of the $NaHCO_3$ buffer solution from pH 7.78 to pH 7.60. This is done by matching the color of the $NaHCO_3$ solution against standard borate-boric acid buffer solutions, adjusted to pH 7.78 and pH 7.60 respectively, also colored with phenolsulphonephthalein.

The temperature was maintained at 20°C., which Chu and Sollmann found most favorable for the normal activity of the turtle heart.

*The immersion fluid.* It was necessary to use a solution which would suppress rhythm in all parts of the heart, with a minimum of injury to the vitality of the cardiac muscle. After a number of preliminary experiments, isotonic sucrose (7.2 per cent) solution was chosen. As demonstrated by Lingle (1901) and subsequently confirmed by ourselves, this

solution does not kill the muscle; for rhythm may be restored even after an immersion of three hours, if the strip is transferred to an appropriately modified Ringer's solution. The apex and sinus-free auricle always remain quiescent in this sugar solution. Sino-atrial preparations practically invariably, and the basal strips often start to beat on immersion in sucrose solution, but they soon cease.

*Selection and preparation of heart strips.* It was decided to make observations upon four regions, namely: the right atrium including the nodal tissue, the base of the ventricle, the apex of the ventricle and the right auricle exclusive of all nodal tissue. These will be referred to later as the sinus-atrium; base; apex; and sinus-free auricle. The strips were prepared as follows:

a. Sinus-atrium. The heart was divided by a cut through the atrio-ventricular junction. The atria were separated by dividing the inter-atrial septum. A third cut through the mouths of the converging venae cavae freed the sinus-atrium. Care was taken that a portion of the veins remained attached to the preparation.

b. Sinus-free auricle. The lateral pouch-like auricle of the right atrium was seized with the forceps and a cut made across the neck of this sac, avoiding the inter-atrial septum.

c. Base. After the ventricle was separated from the atria, the bulb of the aorta and the adjacent muscle were cut away, since this area when left in continuity with the base is at times very resistant to the rhythm-suppressing action of sucrose. A base segment was then removed by a cut horizontally through the ventricle 3 to 4 mm. below its most superior level.

d. Apex. This level was isolated by a horizontal cut through the ventricle 3 to 4 mm. from the tip of the apex.

Owing to variations in the size of the hearts, this method leads to preparations of somewhat unequal size and weight. However, since strips of the same weight from hearts of different size would result in changing the levels (or the relative distance between the segments selected), and since it was the intention to study metabolism at definite levels, the strips were cut of uniform width and appropriate corrections applied for the difference in weight (see appendix B).

*Species of turtles.* The following preparations were used in performing the experiments and determining the average values.

a. Sinus-atrium: ten strips, all from Chrysemys elegans.

b. Sinus-free auricle: six strips all from Chrysemys elegans.

c. Base: ten strips from Chrysemys elegans and one from Chrysemys scabra.

d. Apex: eight strips from Chrysemys elegans, two from Chrysemys concinna, and one from Chrysemys scabra.

*$CO_2$ measurement.* As soon as the heart strip had been removed it was

quickly weighed, washed in isotonic sucrose solution, and immersed in 10 cc. of the isotonic sucrose solution in the tissue tube (corresponding to tube A in the figure of Osterhout's apparatus, page 18 of his article). The circulation of air was started immediately by means of a pump which

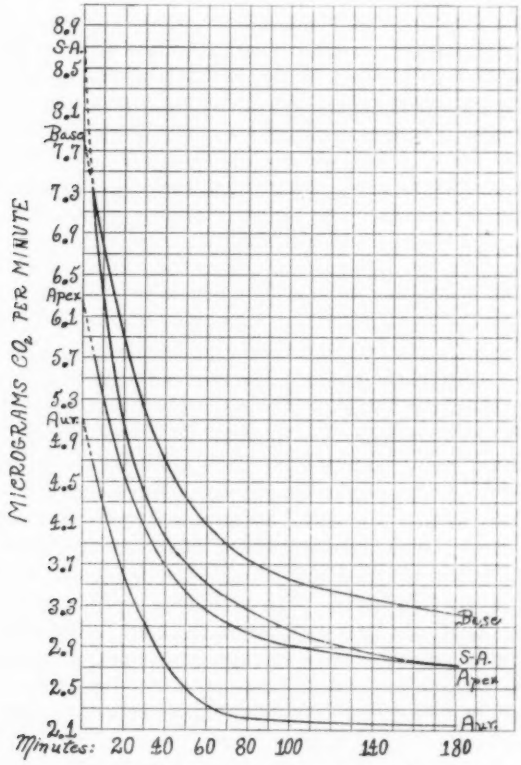


Fig. 1. The metabolic decrement with time

Ordinates. Metabolic rate: The numbers give micrograms per minute for the standard 300 mgm. strip.

Abscissa. Time in minutes since the start of the experiment.

Curves on graph. These are composite curves, constructed from average values, of all individual curves, recalculated to a standard (300 mgm.) weight. The curves are identified thus: *S-A* = sinus-atrium; *Base; Apex; Aur.* = sinus-free auricle.

compresses the bulb "F" in Osterhout's apparatus. The gases were at first made to pass through a tube containing solid NaOH to remove all $CO_2$ from the system. When this was accomplished the stopcocks were turned so as to exclude the NaOH tube, and to circulate the gases evolved

from the tissue through the $NaHCO_3$ solution. The time in minutes required to change the pH of the $NaHCO_3$ solution from 7.78 to 7.60 was noted and is designated as the "time reading."

*Tabulation of results.* The values obtained in the individual experiments were corrected for the weight of the strip (as explained in appendix B), and plotted as smoothed curves against the time (i.e., the time after immersion in sugar solution). From these curves a median curve was constructed for each level of the heart as shown in figure 1. The ordinate in this graph represents the actual $CO_2$ production per minute per 300 mgm. of heart muscle. The method used for converting the "time reading" into micrograms of $CO_2$ is explained in appendix C. The absolute initial value of each curve could not be determined, as it required some time to get the apparatus under way. The probable values, obtained by projection of the curves, are shown by dotted lines.

*Discussion of results.* An examination of the composite curves (fig. 1) shows that the decrement of metabolic rate with time follows the same general type of curve in each of the four levels of the heart. It is seen that the rate of metabolism decreases, at first rapidly; then gradually much more slowly; i.e., as asymptotic curves. The curves for the base and apex are nearly parallel although at different levels. The sinus-free auricle declines more quickly and soon reaches a constant and comparatively low value, showing a more rapid suppression. The sinus-atrium exhibits an especially rapid fall at the start, descending quickly below the curve of the ventricular base and at the end of three hours, approximating the value of the apex curve. The rapid initial fall of the atrial curves (with and without sinus) may perhaps be attributed to the more rapid diffusion of ions from the relatively thin tissue. The prolonged descent of the sino-atrial curve, i.e., the relatively long time before the metabolism reaches a minimum is probably attributable to the high irritability of the nodal tissue proper, which apparently resists the absence of ions longer than the cardiac muscle proper, and which therefore continues to speed the metabolism of the atrial muscle for a considerable time.

Comparing the absolute values, the curves show a quite marked regional gradient in the metabolism of the heart. In the early periods, when the strips are naturally nearest to the normal, the gradient runs: sinus-atrium $>$base $>$ apex $>$ sinus-free auricle; i.e., in the same order as the other previously established cardiac properties. The order of the base, apex and sinus-free auricle is preserved indefinitely; but the metabolism of the sinus-atrium falls progressively. This may be attributed to the complex nature of the sinus strip. The auricular tissue, because of the greater bulk, must be the main source of the $CO_2$ production; but its irritability and metabolic tension are modified by the presence of the nodal tissue. When both tissues deteriorate through the withdrawal of electrolytes, the course of the metabolism must be a complex resultant.

## CONCLUSIONS

Strips taken from different regions of the turtle heart and rendered non-rhythmic by immersion in isotonic sucrose solution, exhibit a definite regional gradient in the rate of $CO_2$ production.

For a certain time after immersion, this gradient is strictly in the order that is characteristic for other properties of the heart: Sinus-atrium > base of ventricle > apex of ventricle > sinus-free auricle.

The basal metabolism slows progressively and rather rapidly after immersion in the sugar solution. The form of the decadence curve varies somewhat for different levels, so that the regional gradient undergoes quantitative changes during this degeneration.

The decadence is relatively faster in the sinus-atrium so that its metabolic rate falls progressively below that of the base and eventually to or below that of the apex of the ventricle.

APPENDIX A. *The apparatus.* This has not been materially changed from that pictured in figure 1 of Osterhout's article (Osterhout, 1918), except that the compression bulb F has been replaced by a flexible rubber tube supplied with valves. A pneumatic "wind-shield wiper" was used as the motor. All glass parts were made of pyrex glass. This is essential in the case of the containers, to prevent alterations in the solutions. Rubber connections and the compression tube were of high grade soft rubber. These as well as the rubber stoppers were thoroughly boiled before using. The valves were constructed by cementing a circular piece of fairly heavy rubber dam over the hole in a rubber stopper in such a way that only about 1 to 1½ mm. remains non-adherent. The authors are indebted to Dr. G. B. Ray of the physiology department of this school for helpful suggestions.

*The buffer solutions.* Carefully prepared boric acid-sodium borate buffer mixtures (Osterhout and Haas, 1918; Clark, 1922) were used, one having a pH of 7.78 and the other a pH of 7.60. Borate buffers are very satisfactory and are moreover quite stable, an obvious advantage. The variable buffer is an M/10,000 $NaHCO_3$ solution. The aim in this case is to secure a solution whose pH may be readily increased or decreased in direct proportion to the concentration of $H_2CO_3$, or in direct ratio to the partial pressure of the $CO_2$ present. The fact that lowering $CO_2$ tension increases the pH is important, for upon this depends the possibility of measuring acidity changes without opening the machine and upsetting the gas equilibrium of the entire system.

*The indicator.* Phenolsulphonephthalein (phenol red) in 0.02 per cent solution (Clark, 1922) was employed as indicator, the quantity being adjusted to meet the requirements of the eye of the experimenter. As a rule 0.3 to 0.4 cc. for 6 cc. of solution is adequate. Color changes were always observed with the aid of a 150 watt daylight lamp in front of which a sheet of thin white paper was placed, to diffuse the rays of light.

*Temperature.* Since temperature influences the metabolism of tissues it was necessary that this factor remain constant. For our purpose 20°C. was selected, as most favorable for the normal activity of the turtle heart. To maintain this, the apparatus was mounted upon a frame and placed in a large glass tank of water constantly stirred by a pump. The tank was provided with a small wire basket attached to the side so that in warm weather ice could be introduced without being permitted to float against the observation tubes.

APPENDIX B. *Calculation to allow for the influence of weight of the strip on $CO_2$ production.* It has been pointed out that the unequal mass of tissue in the different levels of different hearts necessitated the use of pieces of unequal size and surface area. Both of these factors would influence the $CO_2$ production. However, in material of fairly uniform shape, such as our heart strips, the surface and weight would bear a fairly definite ratio to each other, so that either might be used as an approximate index of the other. The weight was of course preferred since its determination is so much easier. The actual relation of the two functions, and especially their relation to metabolism, would be difficult to work out mathematically, but they can be easily plotted as empirical curves, from which the proper factors may then be read.

For the construction of these empirical curves, the strips from each level of the heart were arranged in groups in which the weights of the individual members were fairly close together. The reciprocal "time readings" of the median strip of each group were plotted as ordinates against the weight in milligrams upon the abscissa, thus forming a "weight" curve, which showed the variation in metabolism with change in weight. Since time would have to be represented by a third axis, it could be demonstrated upon a plane surface only by drawing a series of curves for definite intervals of time.

As most of the strips weighed in the neighborhood of 300 mgm., the observations on all other strips were recalculated to this standard. The method is as follows: If 1/R = the reciprocal of the "time reading"; then for any given abscissa value (i.e., on the time axis):

$$1/R \text{ for Y mgm. strip} \times \frac{1/R \text{ for 300 mgm. strip (found from the weight curve)}}{1/R \text{ for Y mgm. strip (found from the weight curve)}} =$$

1/R corrected to the basis of a muscle strip weighing 300 mgm. As an example, a 527 mgm. sinus-atrium strip at ten minutes after the start of the experiment gave a 1/R of 57.0. The average value for a 527 mgm. strip found on the weight curve = 54.5. The average value of a 300 mgm. strip was given by the weight curve as 47.0. Substituting these values in the above general equation, we have: $\frac{47}{54.4} \times 57.0 = 49.2$ or the 1/R which would have been obtained originally had the strip weighed 300 instead of 527 mgm. The corrected figures were averaged, converted into micrograms $CO_2$ (appendix C) and the corresponding $CO_2$ values used as ordinates in constructing the composite metabolism curves (figure 1) for each level of the heart.

The median and range of weight of the preparations from each level of the heart, as used in the experiments, was as follows:

| | |
|---|---|
| Sinus-atrium | 297 (247 to 537) mgm. |
| Sinus-free auricle | 257 (237 to 327) mgm. |
| Base | 452 (237 to 847) mgm. |
| Apex | 302 (207 to 447) mgm. |

As the medians of all but the base are close to 300 mgm., the "corrected metabolism" and the uncorrected medians are practically identical except for the base.

APPENDIX C. *Conversion of the time reading into actual weight of $CO_2$ per minute.* Applying the mass law, as suggested by E. J. Cohn and used by Ray (1924), it is possible to calculate the $CO_2$ production in micrograms per minute, for the standard 300 mgm. strip, by means of the formula: $(CO_2) = (H^+) \times \frac{m}{a}$. In this formula: $(H^+)$ = the hydrogen ion concentration; $m$ = grams $NaHCO_3$ in the 6 cc. of M/10,000 solution $= 5.04 \times 10^{-5}$; $a$ = the dissociation constant of $H_2CO_3$ at 20°C. $= 3.2 \times 10^{-7}$; $(CO_2)$

= grams (or multiplied by $10^6$ = micrograms) of $CO_2$ in 6 cc. of M/10,000 $NaHCO_3$ solution for given values of $(H^+)$. Therefore, $CO_2$ (in micrograms) = $(H^+) \times \frac{(5.04 \times 10^{-5}) \times 10^6}{(3.2 \times 10^{-7})}$. For pH 7.78, where $(H^+) = 0.166 \times 10^{-7}$, this gives 2.60 micrograms $CO_2$; and for pH 7.60 where $(H^+) = 0.251 \times 10^{-7}$, this gives 3.95 micrograms $CO_2$. Consequently to change 6 cc. of M/10,000 $NaHCO_3$ from pH 7.78 to 7.60 requires $3.95 - 2.60 = 1.35$ micrograms of $CO_2$. Since the total volume of the apparatus was 60 cc., it follows that the number 1.35 must be multiplied by 10, giving 13.5 micrograms as the total $CO_2$ evolved by the muscle (assuming an even distribution of $CO_2$ throughout the apparatus at the time of observation). This is divided by the "time reading" to reduce it to $CO_2$ production per minute.

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# THE REGIONAL GRADIENT OF THE TURTLE HEART: ISOTONIC SUCROSE SOLUTION ON TONE, RHYTHM AND CONTRACTILITY

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The preceding paper dealt with the gradient of $CO_2$ production when segments of the turtle heart are rendered non-rhythmic by immersion in isotonic sucrose solution. It brought out two groups of phenomena: 1, That the basal metabolism, shortly after excision, follows the usual cardiac gradient: sinus-atrium > base of ventricle > apex > auricular tissue; and 2, that the basal metabolism of all segments diminishes at first rapidly, then progressively more slowly. The form of these metabolic degradation curves is therefore similar for the different levels of the heart, but it is not identical. Since immersion in sucrose solution also alters the tone and the excitability of the cardiac muscle, it appeared interesting to determine the relations between these changes and the metabolic degradation curves; and further to note whether the irritability and tone phenomena reflect the regional gradient that has been shown to exist for other cardiac properties, as cited in the preceding paper.

The effects of isotonic sucrose solution on the ventricular muscle of the turtle have been investigated by Greene (1898), Lingle (1900), Martin (1904) and others, who found suppression of rhythm and changes of tone, ascribed to the absence of electrolytes in the medium; and recovery after varying periods of time, if the muscle is transferred to electrolyte solutions (Lingle). The effects on the other parts of the heart have apparently not been described; although Eggers (1907) has described the effect of sucrose solutions upon the extra-cardiac nodal tissue of the sinus venosus.

*Experimental method.* Turtles of the species Chrysemys elegans were used exclusively. The segments were cut in the manner described in the preceding paper from the same four regions of the heart, namely: right sinus-atrium, base of ventricle without aortic bulb, apex of ventricle, and left sinus-free auricle. Immediately after excision the strips were attached to a light muscle lever, with a uniform magnification, and suspended in well-aerated isotonic (7.2 per cent) sucrose solution, which was maintained at 20°C. The weight of the lever, although light, placed the muscle under

sufficient tension to favor spontaneous rhythm, which was therefore rather more frequent and more prolonged than with the segments in the Osterhout apparatus. However, all the segments eventually became non-rhythmic before they ceased to respond to electric stimulation. The curves (fig. 1) show that neither the tone nor the height of contraction was affected materially by the presence or absence of rhythm.

The irritability of the muscle was determined by stimulation at intervals, generally of 10 minutes, with a single pair of strong induction shocks from a Harvard inductorium, with two volts in the primary circuit and with the center of the secondary coil at a fixed distance of 8 cm. from the base of the primary coil. This produced maximal contractions, of the same height as spontaneous contractions. In the presence of spontaneous contractions, artificial stimulation was of course unnecessary. The following number of segments were used in the preparation of the data:

| | |
|---|---|
| Sinus-atrium | 7 |
| Base of ventricle | 9 |
| Apex of ventricle | 9 |
| Auricle | 7 |

The tone and height of contraction were measured on the individual tracings, at suitable intervals, and the data for each region reduced to a median curve on the same scale, as shown in figure 1.

*Criteria of excitability.* In comparing the curves of the different levels, the absolute height of contraction should be neglected, since the amount and direction of the muscle fibers vary at the different levels. Attention should be centered on the direction of the curves, and on the time relations. The solid lines show the tone of the relaxed (diastolic) segment. The dotted lines indicate the shortening during contraction, spontaneous (the shaded part of the curves) or by electric stimulation. The meeting of the two lines indicates the point where the muscle ceased to give a well-defined contraction on stimulation. This point may be designated as abolition of contractility, although this applies strictly only to the current used. The muscle at this time may not be absolutely irresponsive to tetanic stimuli, or to stronger single shocks. These stronger stimuli, however, are unsuitable, as they tend to produce persistent alterations in the heart, namely, a sort of veratrine action. The end point of absolute non-contractility is therefore indefinite.

*Diastolic tone.* In the sinus-atrium, the ventricular base and apex, the tone first falls, then rises. Figure 1 shows that these changes follow the normal cardiac gradient, in the sense that the fall is most marked and most persistent in the most irritable part, the sinus atrium; and least marked in the least irritable part, the apex. The rise shows the converse gradient: It comes earliest and is most marked in the apex; least marked in

the sinus-atrium. The behavior of the auricle is anomalous; it exhibits a gradual but progressive fall.

Correlating the tone changes with the metabolism, it may be noted that the sinus-atrium and the auricle, in which the relaxation predominates to the greatest degree, also show a relatively large decrease of basal metabolism. This favors the assumption that the tone and basal metabolism have a causal relation, but without indicating which is cause and which effect.

*Rhythmicity.* The resistance of spontaneous rhythmicity (see the shading in fig. 1) to sucrose solutions shows conspicuously the usual gradient:

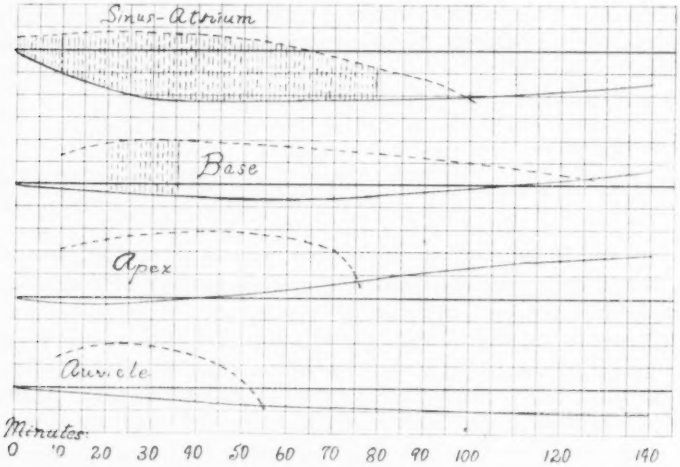


Fig 1. Abscissa: Time after immersion in isotonic sucrose solution. Solid curve: median diastolic tone; broken curve: median systolic tone; intersection of solid and broken curves: median point of inexcitability to standard stimulus; shaded areas: median periods of rhythmicity; sinus-atrium = right sinus-atrium; base = base of ventricle without aortic bulb; apex = apex of ventricle; auricle = left sinus-free auricle.

All sinus-atrium preparations started promptly to contract spontaneously in the sugar solution, and the rhythm endured for 79 minutes, median. With the base, only six strips out of nine started a spontaneous rhythm, but only after a delay of twenty minutes, median, and lasting only 16 minutes, median. With the apex and auricle, none of the strips contracted spontaneously.

*Contractility.* Figure 1 shows that in all parts of the heart the height of contraction in the sugar solution gradually increases for 25 to 45 minutes, and then declines progressively to complete failure to respond to the stimulation. The time when the contractility is abolished shows a definite regional gradient between the base (125 minutes), apex (75 minutes) and

auricle (55 minutes). The sinus-atrium (98 minutes) falls slightly out of series, for according to the gradient it should respond longer than the base. As in the analogous case of the decline of the metabolic gradient, this may be attributed to its compound structure, i.e., to the drag of the less excitable auricular muscle which makes up the main mass of its tissue.

A striking correspondence exists between the median values of basal metabolism at the time when the respective segments become non-contractile. This is practically identical for the base, sinus-atrium and apex, namely, 3.05 to 3.15 micrograms of $CO_2$ per minute, for 300 mgm. of muscle. For the auricle, whose metabolism curve is somewhat more steep, the basal metabolism has fallen somewhat lower, to 2.4 micrograms, before the contractility disappeared. There is therefore a close quantitative relation between the decrease of metabolism and the decrease of contractility; but these observations do not show whether the relation is causal or parallel.

An additional point which should be emphasized is that although the heart strips do not respond to stimulation after a given time, and although their metabolism curves have flattened so as to run more or less parallel to the abscissa, the muscle is not dead; for upon transferring to a modified Ringer's solution (one-half the ordinary amount of potassium and twice the amount of bicarbonate) good recovery resulted even after an immersion of three hours in sucrose solution. Atrial strips are more susceptible and their recovery while good is not usually as permanent as in the ventricular preparations which have been known to maintain their regained rhythmicity for as long as forty-eight hours. The presence of bicarbonate is necessary for prolonged recovery, for 0.7 per cent NaCl, and even alkali-free Ringer's solution permit only a temporary revival.

## CONCLUSIONS

The characteristic changes in the tone, rhythmicity and contractility of cardiac muscle which result from immersion in isotonic sucrose solution reflect the typical regional gradient of sinus-atrium > base > apex > auricle, with a few minor complications, and they correspond closely with the changes in basal metabolic rate.

Of the tone changes, the primary fall is most marked with the sinus-atrium, the secondary rise with the apex. Spontaneous rhythmicity is most resistant in the sinus-atrium, and decreases with the gradient. The persistence of response to stimulation, after the muscle has become non-rhythmic, also decreases in the general direction of the gradient, and in close parallel to the decline of basal metabolism.

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# THE REGULATION OF THE FLOW OF BILE

## II. Effect of Eliminating the Sphincter of Oddi

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In a previous paper (1925) evidence was presented indicating that the resistance of the so-called sphincter of Oddi had probably best be attributed to the tonicity of the duodenum. The hypothesis, that the regulation of bile flow is dependent upon intra-abdominal pressure and tonicity and peristalsis of the duodenum, was suggested. It was shown that when the resistance in the duct was estimated by cannulizing the duct and connecting it with a pressure bottle it varied directly with the tonicity of the intestine. The normal resistance varied considerably depending upon the condition of the animal and the state of anesthesia. Invariably when pilocarpine or eserin was given to increase the tonus of the intestine, the duct showed increased resistance. On the other hand following atropin or adrenalin, bringing about relaxation of the intestine, the resistance fell.

If the duct may be said to have a sphincter, anatomists agree that it lies near the opening at the papilla of Vater. Meltzer (1917) suggested that the sphincter had a reciprocal innervation with the gall bladder. This innervation may be so closely linked to the intestine that it is impossible to study the nervous mechanism of the sphincter separately. However, if the end of the duct wherein the sphincter lies could be eliminated and other mechanical relations kept normal, a way would be open for investigating the influence of the tonicity of the intestine upon this resistance. Likewise, if the influence of the contraction of the circular musculature of the intestine in that section where the duct is imbedded could be eliminated, the resistance in the duct would be uncomplicated.

Throughout the experiments dogs were used. Early in the work attempts were made to free the duct from the duodenal musculature, leaving only a direct entrance of the duct into the intestine with the sphincter intact. It was found that such an operation was of doubtful value. There invariably followed many adhesions. The duct is naturally pulled against the torn surfaces and held there. Some doubt also exists as to when the dissection has reached near enough the sphincter. One-fourth inch of the intestine with good circular muscle fibers might complicate the situation

when resistance in the duct is later measured. The nerve supply may be irreparably mutilated and the sphincter unable to do itself justice.

The other possibility remained, namely, to eliminate the sphincter and see whether or not an oblique entrance of the duct through the intestinal musculature would afford resistance. At first it was thought feasible to dissect out the intra-mural portion of the duct to within about one-fourth

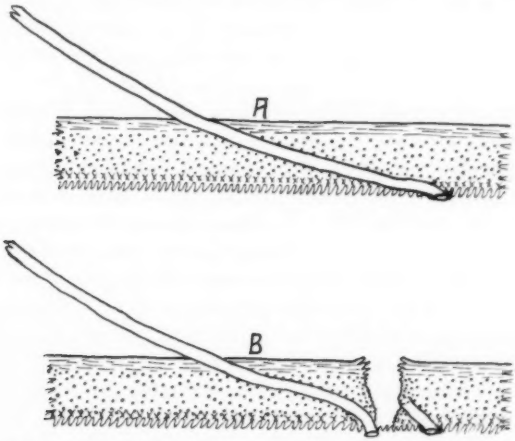


Fig. 1. Drawings to illustrate *A*, the manner in which the cystic duct enters the duodenal wall, and *B*, the operation used to exclude the sphincter of Oddi.

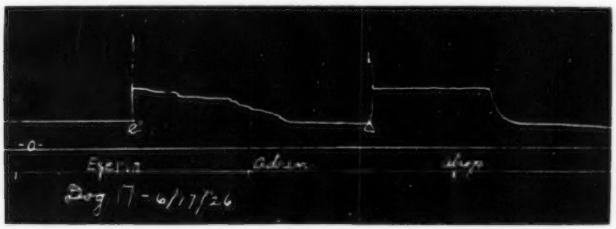


Fig. 2. Normal resistance in duct (where the sphincter of Oddi has been excluded by operation) is 8 mm. Hg. Eserin intravenously increased the resistance withstood to 18 mm. Adrenalin and atropin caused it to fall again.

inch of its opening, section and transplant it obliquely into the duodenum at a new site. This operation was found not to be the most desirable for our purpose. It was then determined to dissect down to the duct at a point just back of the papilla, without tearing the musculature too much, and section it. An opening was then made in the mucosa and the proximal end of the duct turned into the lumen of the intestine (fig. 1). One or two stitches only were required to close the wound in the intestine and the

duct was undisturbed throughout the greater part of its intra-mural portion. The end containing the sphincter remained in place.

This exceedingly simple operation permitted all that could be asked. Allowing six to eight weeks for complete recovery the resistance in the duct was tested in the usual way. An average normal resistance was shown and the response to eserin intravenously increased it as the tonus of the intestine increased. When adrenalin or atropin was given the resistance decreased with the decrease in tonicity of the intestine (fig. 2). The resistance found in these animals was not different from that seen in normal animals.

Discussion. Kodama (1926), working in Graham's laboratory, emphasizes the elasticity of the gall bladder as a factor in bile flow. He states, "The elasticity of the gall bladder wall and the inside pressure of the lumen of the gall bladder are perhaps the most important factors in the flow of the gall bladder bile. Increased intra-abdominal pressure is an important auxiliary factor. The contents of the gall bladder are removed by the opening and closing of the sphincter of Oddi." In a footnote he says, "The term 'sphincter of Oddi' has been used in this article to represent the mechanism of closure of the ampullar end of the common duct, but in a later paper now in press, evidence will be given to show that tonus of the duodenal musculature is perhaps even more important than the so-called sphincter of Oddi." This indicates he has found evidence that confirms the work reported earlier from this laboratory (Burget, 1925).

Boyden's (1925) observation of the gall bladder as it becomes empty would tend to exclude any major activity on its part in evacuation of its contents except, as he states, when it is distended elasticity may be a factor.

Winkelstein and Aschner (1926) speak of an ebb and flow of bile between the gall bladder and common duct when the sphincter is closed caused by the variations in intra-abdominal pressure with respiration. They further state, "When the sphincter of Oddi is somewhat relaxed by the gastric chyme or innervation changes the intra-abdominal pressure at inspiration apparently squeezes bile from the gall bladder into the duodenum. Neither a reciprocal action between the sphincter of Oddi and the gall bladder nor sufficient contractile power of its musculature to empty the gall bladder was found in our experiments." Evidently they have given no consideration to the tonicity and peristalsis of the duodenum which factors I (1925) have shown to be important. Further, in the work here submitted the sphincter of Oddi may be entirely excluded and the flow of bile continue normally from a normal duct.[1]

In an earlier article (Winkelstein and Aschner, 1924) the same authors using pilocarpine, adrenalin and atropin subcutaneously found the first

[1] Normal except for operation as described.

to increase the resistance in the duct and adrenalin and atropin to decrease it. They speak of this action as being on the sphincter of Oddi. I have shown (fig. 2) that the same results obtain when the sphincter is excluded. Therefore, such an assumption is no longer warranted or at least not needed.

Whitaker and Boyden (1926) have found that following an egg yolk and cream meal the emptying of the gall bladder takes place periodically. They believe this is due to rhythmic contractions of the sphincter of Oddi and peristaltic waves over the ducts. Injections of pituitrin were found to revive this rhythm after it had disappeared.

Aschoff (1924) states, "All these distal bile passages (distal cystic duct, hepatic and common ducts) have practically no smooth muscle, being built from only connective tissue and elastic fibers and are very rich in specific glands." Hendrickson (1898) found only a small amount of muscle fibers scattered amongst much connective tissue. Matsuno's (1923) work is in entire accord on this point. Burden (1925), speaking of the musculature of the ducts, calls it a "loose network."

It seems probable, then, that this periodicity corresponds to intestinal peristalsis and rhythmic variations in tonus of the duodenum. The pituitrin would certainly induce activity of the intestine. Such activity has been shown to cause a flow of bile when there is any pressure in the duct (Burget, 1925). Robins and Boyd (1923) have shown that fats depress the tonicity of the gastro-intestinal tract. Thus a lowered tonus in the duodenum would account for bile beginning to flow from a filled gall bladder fifteen minutes after a meal of egg yolks and cream.

In explaining what force expels the bile from the gall bladder, Whitaker and Boyden (1926) found that following intravenous injection of pituitrin or barium chloride, bile begins to flow in three minutes. This would be expected from induced intestinal peristalsis. They say, "complete emptying depends upon opening of the sphincter of Oddi, for without this action the gall bladder does not discharge its contents. Conversely, when the sphincter papillae is eliminated by an inlying cannula, the gall bladder does not fill." They do not explain but no doubt the inlying cannula extended through the intra-mural portion of the duct. It is difficult to make sweeping interpretations from such experiments. In view of the anatomical findings, it seems more plausible to account for the rhythmic flow through activity of the duodenum than through any action on the part of the ducts and sphincter.

Magnesium sulphate caused a moderate reduction in size of the gall bladder as seen by Whitaker and Boyden (1926). Wakerlin (1926) has shown that magnesium sulphate when placed in the duodenum caused it to relax in twelve out of fourteen experiments. The tonicity of the intestine was registered by an inflated balloon placed in the lumen and connected with a water manometer.

Using oleic acid, McClure, Mendenhall and Huntsinger (1925) obtained a similar flow of bile to that produced by magnesium sulphate. It seems probable that oleic acid would depress the tonus of the intestine and allow a flow in the same manner as the fats used by Whitaker and Boyden (1926).

## CONCLUSIONS

1. Drugs that affect the tonus of the intestinal musculature affect the resistance encountered in the duct to the same extent in the absence of the sphincter of Oddi as when the sphincter is intact.

2. The sphincter of Oddi is probably not at all concerned in the regulation of the flow of bile, at least in those animals where the duct enters the duodenal musculature obliquely.

3. A reciprocal activity between the gall bladder and the sphincter that would influence the flow of bile is improbable.

4. The tonicity of the duodenal musculature, where the duct enters it obliquely, constitutes a sphincter-like mechanism that prevents in the presence of a gall bladder a continuous flow of bile.

5. Further evidence is presented indicating that bile flow is regulated by intra-abdominal pressure and tonicity and peristalsis of the duodenum.

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# THE EFFECTS PRODUCED ON THE WEIGHT, RESPIRATION AND TEMPERATURE BY ADDING DESICCATED WHOLE ADRENAL GLAND AND DESICCATED ADRENAL CORTEX TO THE DIETS OF NORMAL PIGEONS

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In the course of administration of extracts of the suprarenal glands to pigeons, a considerable loss of body weight occurred. It therefore appeared interesting to determine whether this reaction was caused by the medullary or cortical portion of the gland, whether it was due to a specific constituent, and to study the physiological mechanism of the weight loss. The changes of the respiration rate and body temperature were also studied since we found that these functions bear a definite relation to malnutrition (Barlow, 1926).

METHODS. The experiments were carried out on adult pigeons ranging in weight from 280 to 500 grams, the medium being 340 grams. The normal diet consisted of a standard mixed whole grain pigeon food containing grit. All of the pigeons were allowed free access to both food and water at all times during the period of study and maintained themselves at a constant level throughout the 6 weeks of preliminary observations. These observations were repeated at approximately 3-day intervals during the period of adrenal gland feeding and until the recovery of the body weight level had been complete.

The experimental procedures shown in table 1 were as follows: Group A, 20 birds were maintained on a normal diet as controls throughout the 45-day period of study; group B, 27 birds were maintained on a normal diet plus 0.25 gram of desiccated whole adrenal gland in gelatin capsules per day from the 1st to the 30th day; group C, 26 birds were maintained on a normal diet plus 0.25 gram of desiccated adrenal cortex in gelatin capsules per day from the 1st to 30th days; group D, I, 4 birds were maintained on a normal diet plus 0.001 gram basic adrenalin in milk sugar per day from the 1st to 10th days; group D, II, 5 birds were maintained on a normal diet plus 0.003 gram basic adrenalin from the 1st to 10th days; group E, 16 birds were held on a complete fast with access to water; group F, 8 birds were maintained on a normal diet plus 0.25 gram of milk sugar in gelatin capsules from the 1st to 30th days.

The weight, body temperature, respiration rate and the individual food intakes of groups D, I and II, were observed every 24 hours instead of every 3 days as with all other groups of table 1.

RESULTS. The massed data for each group of pigeons have been medianed. Since the respiration and temperature changes were observed with only those birds on a complete fast and a number of those birds on whole adrenal gland and adrenalin therapy respectively, only the weight curves have been plotted, figure 1. The curves shown by figure 1 correspond by letter to the respective groups of pigeons in table 1.

The control pigeons of group A show an approximately constant level throughout the period of study. The administration of milk sugar in gelatin capsules had no demonstrable effect on the second control group, F.

TABLE 1

| GROUP | NUMBER OF BIRDS | DIET | DRUG ADDED | DOSE MILLIGRAMS PER DAY | PERIOD DAYS | | |
|---|---|---|---|---|---|---|---|
| | | | | | Drug | Recovery | Total |
| A | 20 | Normal | None | None | 0 | 0 | 45 |
| B | 27 | Normal | Desiccated whole adrenal gland | 0.25 | 30 | 15 | 45 |
| C | 26 | Normal | Desiccated adrenal cortex | 0.25 | 30 | 15 | 45 |
| D, 1 | 4 | Normal | Adrenalin (base) in milk sugar | 0.001 | 10 | 5 | 15 |
| D, 2 | 5 | Normal | Adrenalin (base) in milk sugar | 0.003 | 10 | 5 | 15 |
| E | 16 | Complete fast with access to water | None | None | 0 | 0 | 33 |
| F | 8 | Normal | Milk sugar | 0.25 | 30 | 15 | 45 |

The pigeons receiving desiccated whole adrenal gland, group B, show a very definite depression of body weight throughout the period of adrenal administration and a 25 per cent fatality. The character of the individual changes is shown in figure 2. The weight depression became evident with the first observation taken, i.e., on the 3rd day after the adrenal feeding was begun and increased progressively until the 18th day. The median weight level thereafter, although remaining constant until the 30th day, is only approximate as the fatalities developed during that period.

The recovery of the surviving pigeons, on cessation of the adrenal gland feeding, was a gradual one and was approximately complete at the 15th day.

The response of the pigeons to whole adrenal gland administration is in contrast to the response of group C to the administration of desiccated

adrenal cortex—figure 1. The weight curve of group C corresponds very closely to that of the control group A and the individual variations, as

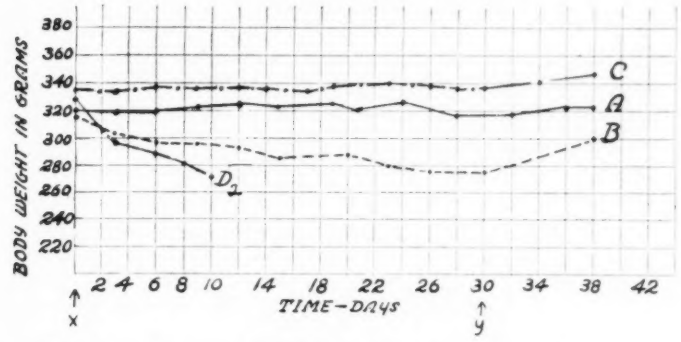


Fig. 1. The several curves labeled *A*, *B*, *C* and $D_2$ represent the medians of the massed data for the groups designated by the corresponding letters in table 1. The experimental feeding time extending from *X* to *Y*.

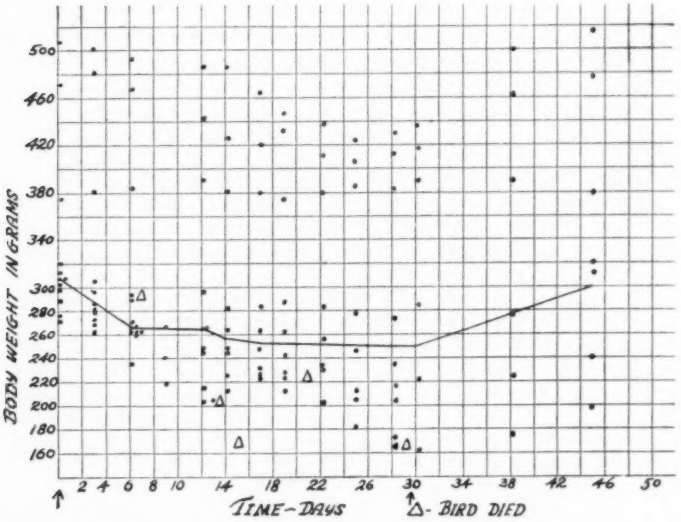


Fig. 2. Represents the absolute change of body weight under whole adrenal gland therapy group B.

noted in figure 3, were negligible throughout the period of study and demonstrate clearly that the toxicity of the gland is due to the medullary rather than cortical portion.

The oral administration of basic adrenalin to pigeons in a dosage corresponding to the adrenalin content of the desiccated whole adrenal gland has only a small depressant effect on the weight, respiration and temperature of group D, I. The effect of increasing the daily dose of adrenalin to

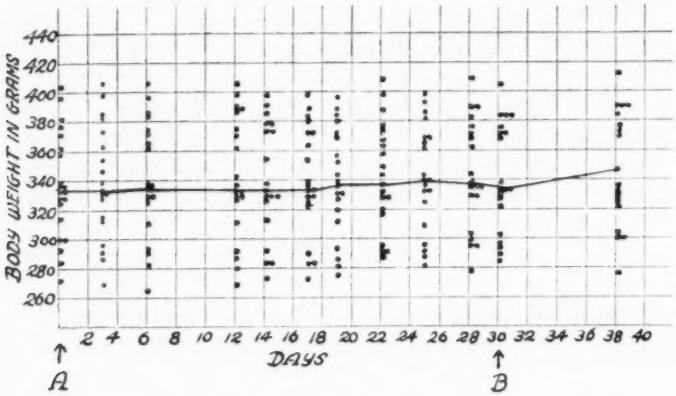


Fig. 3. Represents the absolute body weight changes observed with group C under adrenal cortex therapy. Time of feeding, *A* to *B*.

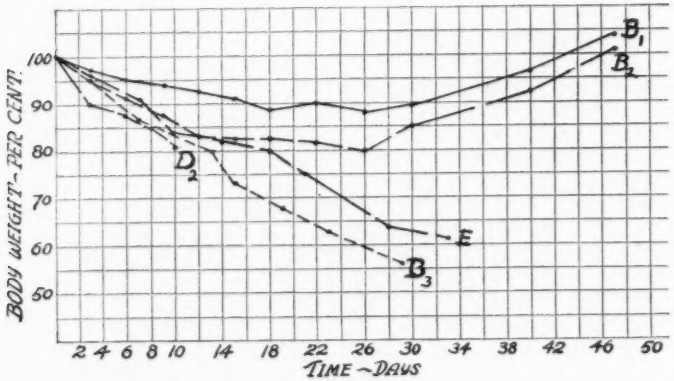


Fig. 4. Curves $B_1$, $B_2$ and $B_3$, represent the medianed data for all birds of group B above 340 grams weight; all birds below 340 grams weight, and all birds dying under whole adrenal therapy.

Curve $D_2$ represents the medianed body weights of those birds of group $D_2$ receiving 0.003 gram basic adrenalin daily.

Curve *E* represents the medianed body weights of those birds of group E—under a complete fast with access to water.

3 mgm. is shown in figure 1 as curve $D$. The depressant effect of this concentration was quite evident on the weight, respiratory rate and temperature. This response paralleled that of those pigeons dying under the administration of desiccated whole adrenal gland, both in direction and degree, although the adrenalin dosage was not continued after the 10th day.

Since we have observed that a condition of malnutrition is accompanied by a parallel depression of the body weight, respiration rate and body temperature (Barlow, 1926), and particularly since the food intake of those pigeons receiving pure adrenalin was observed to decrease to approximately zero, it was deemed advisable to compare the weight response of those birds dying under whole adrenal gland administration with that of birds on a complete fast and to that of birds on a voluntary complete fast due to the administration of adrenalin, with the idea of determining the mechanism of the reaction, figure 4. The curves $B_1$ and $B_2$ represent the percental body weight of the large and small birds, i.e., above and below 340 grams, respectively, of group B. The difference is only quantitative, since a number of the smaller birds shown by curve $B_3$ died under whole adrenal therapy. Curves $D_2$ and $E$ represent the median percental body weights of those birds receiving 0.003 gram basic adrenalin daily and those birds on a complete fast, respectively.

The parallelism in direction and degree of the curves $B_3$, $D_2$ and $E$ is quite marked and since the weight loss of the birds represented by the curves $D_2$ and $E$ is quite close, and the weight loss of the birds represented by the curves $D_2$ and $E$ is directly explained by the known diminished food intake, it seems logical to ascribe the similar loss of weight of those birds dying under adrenal therapy, $B_3$, to the same cause. A more logical comparison can be made by the relative losses of body weight of groups $B_3$ and $E$ respectively. Thus it may be seen that the median terminal weight loss of those birds dying under adrenal gland therapy was $-33$ per cent, while that of those birds dying from starvation was $-47$ per cent. The figures are of the same order of magnitude, therefore the main cause of death was starvation. The difference might indicate some direct toxic action of epinephrine but due to the small number of birds used the results probably fall within the range of error.

Discussion. Chidester (1912) observed that the body weight of fowls decreased during a 10-day feeding with desiccated whole adrenal gland, the maximal loss being 15 per cent.

McKinley and Fisher (1926) likewise have shown a toxic reaction of the white rat to whole adrenal gland administered by mouth. The effects noted were produced by the medullary rather than the cortical portion as shown by our experiments.

Protein as such does not produce the reactions noted since the cortex gave no change under any conditions studied.

The fat extractives do not show these toxic effects on the rat as noted by McKinley.

The choline content of the gland would act as a direct antagonist to any adrenalin depression on the alimentary tract thus tending to flush the canal by stimulating peristalsis.

The potassium content, although quite high in relation to body fluids, is negligible when the potassium content of the various foods is considered and especially in the dosage used.

The adrenalin constituent is the only principle which gives effects quite similar to the stronger manifestations of whole adrenal therapy. Rowntree (1924) noted a marked gastric disturbance as a result of the oral ingestion of whole adrenal gland. The author has noted the same reaction in dogs and pigeons and confirmed Rowntree's observations on man, consequently, adrenalin by mouth, in a dosage analogous to the epinephrine content of the ingested gland material gives the same gastric and psychic disturbances noted for the whole gland preparations, i.e., nausea, vomiting, diarrhea, peripheral dilatation and a small rise in blood pressure (Hitchcock, 1924).

McKinley and Fisher failed to obtain a reaction to adrenalin in huge doses with the rat. Likewise, they observed that adrenal glands which had been "adrenalinized" i.e., freed of their adrenalin content gave a reaction, when fed to the white rat, similar to that obtainable with the untreated glands. The latter test, however, because of its nature may be discounted.

## CONCLUSIONS

The gastric administration of desiccated adrenal gland to pigeons results in anorexia, gradual loss of body weight, slowing of respiration, fall of temperature and frequently termination in death; the curves of these changes being identical in both direction and degree with those of fasting of corresponding degree. These effects are due to epinephrine, and can be reproduced by the oral administration of the isolated alkaloid in dosages approximating those given with the gland. They result from the disturbance of gastric functions, presumably by sympathomimetic stimulation. The adrenal cortex is not concerned in these effects.

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# CRETINISM AND MYXEDEMA PRODUCED EXPERIMENTALLY IN SWINE

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No distinct advances were made with regard to the diseases due to deficiency of thyroid until it became known that some of them could be reproduced by thyroidectomy in certain of our laboratory animals. However, an animal entirely satisfactory for such experimentation was not found. Dogs and cats do not lend themselves well to thyroidectomy because their thyroid and parathyroid glands are so intimately associated that successful operation is difficult. Guinea pigs, rabbits, rats and mice are too small for easy operation and satisfactory observation. We have found that swine are excellent animals for experiments on the thyroid; they are easily procured, they lend themselves well to surgical procedures, and results are quickly obtained.

The neck and thorax of several swine were carefully dissected and all glandular tissue found was examined microscopically to determine the extent and distribution of the thyroid, accessory thyroid and parathyroid glands. A detailed study of the blood supply and anastomosis of the thyroid was made by dissection and by roentgenograms taken after the injection of the thyroid artery with barium-gelatin solution and lipiodol (Gross, 1921). Poland China and Duroc swine were the breeds used in these experiments.

Some of the common animals which have been used for the study of the thyroid gland are the rabbit, guinea pig, dog, goat and sheep. Mossou, in 1892, removed the thyroid from many animals, including a horse, donkey, dog, goat, deer, steer, rabbits and two swine, one adult and one suckling pig. In the adult swine no change was noted two months after operation; the suckling pig while nursing grew at the same rate as his litter mates. However, eighteen days after this animal was weaned there were many more noticeable changes in it than in the others. There was no increase in stature, the hair had grown long and coarse, the voice had become hoarse, and the body was rounded and widened. When the animal was about two and one-half months of age it died. At necropsy edema of the subcutaneous tissues of the neck and forelegs was noted.

Basinger (1916), working with rabbits, was able to obtain experimental cretinism in approximately 60 per cent of his animals. Liddell and Simpson (1925, 1926), working with sheep and goats, produced typical cretinism by removal of the thyroid gland. Munk (1897) removed the thyroid from dogs, cats, monkeys and rabbits, with positive results. Haushalter and Jeandelize (1902) removed the thyroid from many animals, including

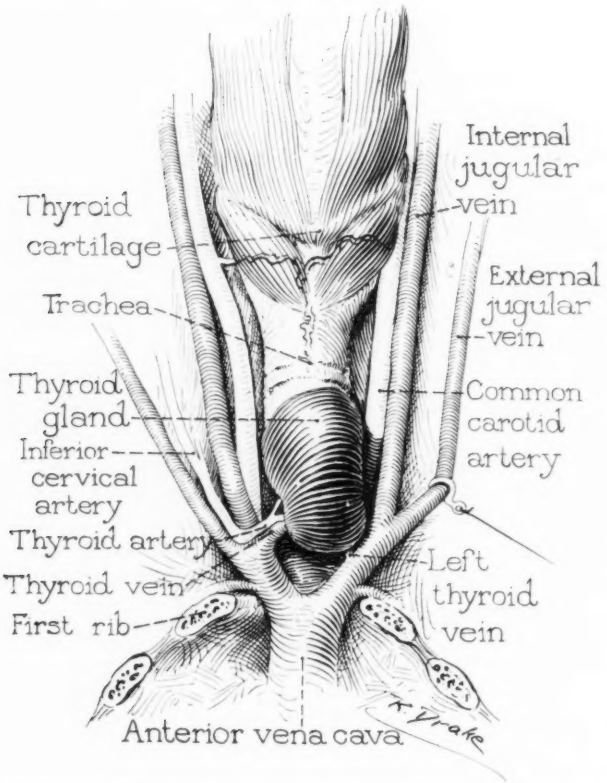


Fig. 1. Position of the thyroid gland and its relation to the adjacent blood vessels

lambs, cats and rabbits, but had many failures. Many of the workers experienced difficulty in getting a high percentage of successful results from thyroidectomy. However, the unfavorable results may have been due to the fact that many of the experiments were performed on carnivora in which the parathyroids are so intimately associated with the thyroid that removal of this gland is difficult without removal of, or injury to the parathyroids (Welsh, 1897-98).

In swine the thyroid gland is situated on the ventral surface of the trachea in the median line, just anterior to the thoracic aperture in the angle formed by the carotid arteries and jugular veins (Sisson, 1917). It is composed of two lateral lobes fused in the median line to form a single organ (fig. 1). Accessory thyroid glands, if present, are small pink bodies usually immediately adjacent to the main gland, although in one animal we found a small accessory thyroid within the thorax, dorsal to the vena hemiazygos. The blood supply of the thyroid gland is quite

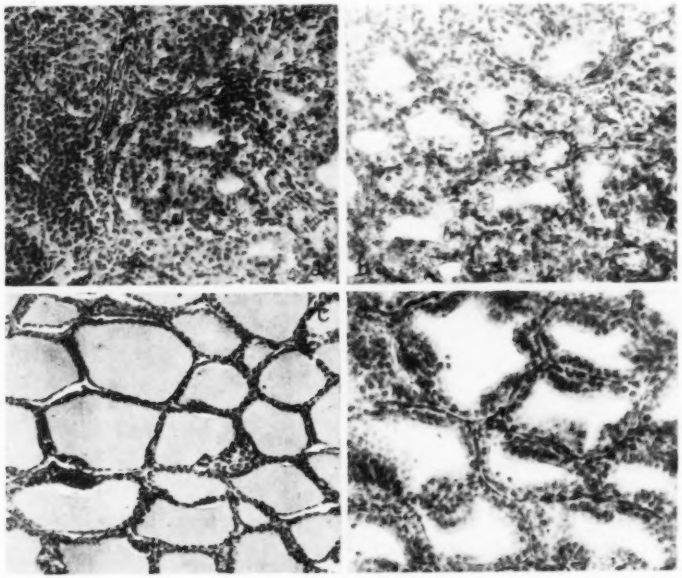

Fig. 2. *a*. Parathyroid cells in an accessory thyroid gland (× 120). *b*. Thyroid gland of young swine. The alveoli are small, irregular and contain little colloid. Some of the acini are lined by columnar epithelium (× 150). *c*. Thyroid gland of a swine seven months old at the time of thyroidectomy (× 120). (Compare with fig. 2 *b*.) *d*. Accessory thyroid gland illustrating the marked hypertrophy following removal of the original thyroid gland (× 150).

simple, the main arterial supply being through a single thyroid artery on the right. This is a branch of the inferior cervical artery. The chief arterial anastomosis of the thyroid gland is with the posterior laryngeal arteries, which are branches of the laryngeal arteries and arise from the carotids (Baumgartner, 1924; Sisson, 1917) (fig. 1). These observations on the circulation were confirmed by roentgenograms taken after the injection of the thyroid artery with barium-gelatin solution and with

lipiodol. By this method we were also able to demonstrate an anastomosis between the arterioles within the thyroid and those of the perithyroid tissues. The venous return is usually through two thyroid veins, one on each side. These empty into the right and left deep jugulars. The right thyroid vein is frequently the larger of the two; it arises near the posterior pole and proceeds posterolaterally, emptying into the deep jugular vein near its juncture with the superficial jugular vein. In none of our dissections have we found any definite parathyroid bodies. The only parathyroid cells identified by us were in accessory thyroid glands (fig. 2a). According to Ellenberger (1906) the parathyroid bodies of swine are situated in the region of the bifurcation of the carotid arteries, either in the thymus or adjacent adipose tissue, or sometimes in both places. Forsyth (1908) dissected a collared peccary (a suidae or pig) but was unable to demonstrate parathyroid glands.

Two groups of swine were used in our experiments. In the first group were Poland China and Duroc breeds, litter mates from two litters. These pigs were procured at the time of weaning, when about six weeks of age, and weighed from 4.4 to 10.5 kgm. In the second group were Duroc swine about seven months of age, weighing from 50 to 60 kgm.

Technic of experiment. The animals were fasted for twelve hours before operation. They were anesthetized with ether in an ether cabinet (Mann, 1920), then after they were made secure on the operating table in the dorso-recumbent position, the anesthesia was continued with the ether cone. The ventral surface of the neck was scrubbed and shaved from the angle of the jaws to the pectoral girdle and the skin prepared with benzin and iodin in the usual way. A median-line incision was made through the skin and subcutaneous tissues down to the muscles. This incision extended from the posterior margin of the larynx to a few centimeters anterior to the manubrium sterni. Then by blunt dissection the bodies of the sterno-cephalicus and sterno-thyro-hyoideus muscles were separated in the median line and retracted laterally, the space occupied by the thyroid gland thus being exposed. This blunt dissection was continued until the thyroid gland was freed from the surrounding fascia, fat and adjacent thymus, and until the thyroid vessels were exposed. The vessels were then clamped, the thyroid extirpated and a gauze pack inserted in the space formerly occupied by the gland. After a minute or two this pack was removed. Usually complete hemostasis was afforded. However, if bleeding persisted the bleeding vessels were ligated. We have also removed the thyroid gland in swine by simply freeing the gland from the surrounding structures and then picking it up in the fingers and crudely twisting it off. The insertion of a gauze sponge for a few minutes made hemostasis complete. After bleeding had ceased, a careful search was made for accessory thyroids near the site of the

main gland; to make certain that no thyroid tissue was left, we always removed all visible lymph nodes and other glandular structures. The accessory thyroids were from 1 to 3 mm. in diameter. The size of the main thyroid gland varied with the age and weight of the swine; in the first group the glands weighed from 1.1 to 2.4 grams. After all glands had been removed, the wounds were closed by suturing the skin with catgut and covering it with collodion.

All the animals including the controls were on the following ration: ground corn, 35 pounds; ground oats, 25 pounds; wheat middlings, 35 pounds; and oil meal, 5 pounds. This ration has a nutritive ratio of 1 part of protein to 5.46 of carbohydrate (Henry and Morrison, 1917). The swine were allowed all the food they would eat and were fed twice daily.

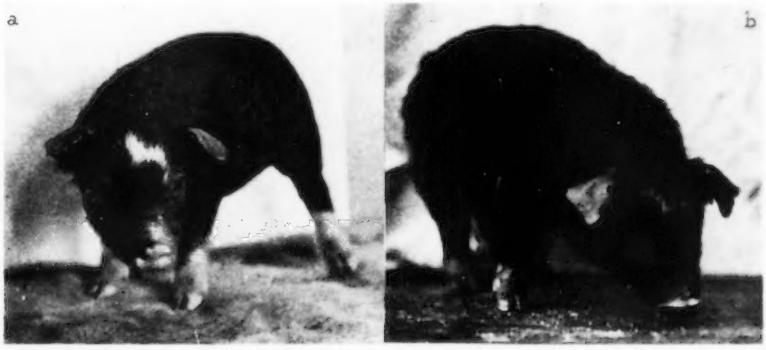


Fig. 3. *a*. Immediately before thyroidectomy. The alert expression is apparent. *b*. Thirty-one days after thyroidectomy. Note the excelsior-like hair, drooping ears, stupid expression, fullness of the neck and forelegs, pot belly and wrinkled face.

Results. In any study of animals after removal of the thyroid, the age at which thyroidectomy is performed is important in the analysis of results. The most striking changes are noted when the gland is removed before puberty (Hammett, 1926). The age of puberty in swine is approximately six to seven months.

In the first group the first change was in the character of the hair, noted between ten days and two weeks after thyroidectomy. The hair became harsh, dry and excelsior-like, and appeared to have grown longer than usual. Other changes came on in rapid succession: the voice changed so that the squeals were slow, hoarse and deliberate, and there was wheezing on inspiration. The general form of the animals changed; they grew pot-bellied, wrinkle-faced, thick in the neck and legs, and puffy in the hocks. Their ears dropped and the animals were dull, lethargic and inert (fig. 3). The control swine remained mentally alert, held their ears erect,

showed curiosity, and their squeals were sharp and piercing. Their bodies remained smooth and slender, and the hair, although wiry-looking, felt soft and pliable. These changes were more noticeable in the Poland China swine. The apparent rate of growth, as indicated by weight, was about the same in both the test and control animals (fig. 4), but the absolute rate of growth of the test swine was less than that expected for animals of the same age and size. Hammett has made the same observations in rats.

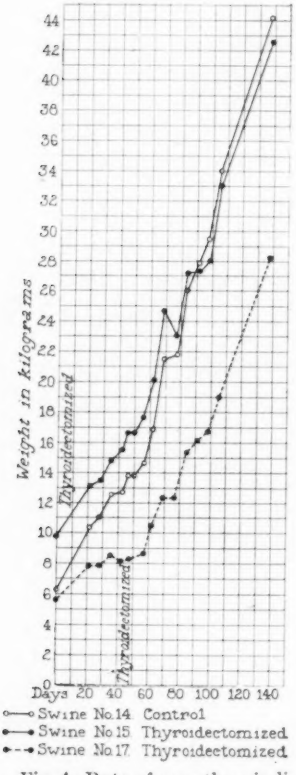


Fig. 4. Rate of growth as indicated by the weights of thyroidectomized and control animals.

The swine in the second group (which had reached the age of puberty) showed less striking symptoms of thyroid deficiency than those in the first group. The hair became curly and dry, and in one animal most of it came out. Growth was retarded. These swine underwent the same change of attitude and disposition as those in the first group: they became lethargic and stupid, and lost their curiosity.

The thyroidectomized swine apparently were hypersusceptible to disease. From thirty-two to forty-four days after operation, when symptoms of thyroid deficiency were well established, four swine developed mild ulcerative stomatitis and within a few days died from pneumonia. These swine had been kept in the same pens with the control animals, yet the controls remained healthy and normal.

The thyroid in the young animals was composed of small acini lined by tall columnar epithelium (fig. 2 *b*); in the older animals the acini were filled with colloid and lined with cuboidal epithelium (fig. 2 *c*). However, there were some areas which suggested the fetal type of thyroid.

The accessory thyroid glands removed at the time of thyroidectomy had approximately the same appearance as the thyroid gland, except that some of them contained parathyroid cells (fig. 2 *a*). Those accessory thyroids inadvertently left intact during thyroidectomy when subsequently removed showed the same structure as the hyperfunctioning thyroid in the human being with exophthalmic goiter; all of the par-

enchymatous elements were hypertrophied. The acini were irregular, lined with columnar epithelium, and revealed little if any colloid (fig. 2 *d*).

If the expected changes were not evident within three weeks after thyroidectomy an exploratory operation was performed on the neck. In all such cases hypertrophic accessory thyroids were found near the site of the original gland. One swine developed typical symptoms of thyroid deficiency within eighteen days after operation, then rapidly started to recover. On exploring his neck at the end of thirty-one days, we found an accessory thyroid weighing 1.9 grams. The original gland had weighed only 1.1 grams. In only one animal did we have complete failure. This animal was explored once and bits of accessory thyroids were removed, but no signs of thyroid deficiency developed. At necropsy careful dissection of the neck with microscopic examination revealed many small hyperfunctioning accessory glands.

Discussion. From these observations it is evident that swine are excellent animals for the production of experimental cretinism and myxedema. The gland lends itself to easy removal because of its location, simple blood supply, and the fact that it is a single organ. Accessory thyroids, when present, because of their location are easily removed. The coagulation time of swine blood is so short (one and one-half to two and one-half minutes) that there is little annoyance from hemorrhage. Very striking symptoms of thyroid deficiency appear promptly following thyroidectomy.

No parathyroid tetany developed in any of the animals operated on, and this fact shows that one of the frequent causes of mortality following thyroidectomy can be obviated. Another item of importance is that the swine is one of the few omnivorous domesticated animals available.

The age at which thyroidectomy is performed is an important consideration. The younger the animal at the time of thyroidectomy the more marked the immediate influence of the extirpation on the rate of growth as indicated by the weight, but because of the inherent tendency of young animals to grow the rate of growth of the test group, as indicated by the weight, tends to become parallel with that of the control animals and about equals it in amount (Hammett, 1926) (fig. 4). The older the animal at the time of extirpation, the less pronounced is the final effect.

The hypersusceptibility to disease of the thyroidectomized swine may be due to a lowered resistance and accompanying lowered metabolic rate.

## SUMMARY

Swine are very satisfactory animals for the production of experimental cretinism and myxedema, because of their peculiar anatomy and physiology. They responded quickly to thyroid deficiency with definite symptoms. Distinct parathyroid glands were not found in swine, but islands

of parathyroid tissue were demonstrated in accessory thyroid glands. Parathyroid tetany did not develop in swine following complete thyroidectomy. The age at which thyroidectomy was performed was of importance. The animals which were operated on before puberty showed a more marked reaction. Swine from which the thyroid had been removed, apparently were hypersusceptible to disease.

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# DEPOSITION OF DYES, IRON AND UREA IN THE CELLS OF A RENAL TUBULE AFTER THEIR INJECTION INTO ITS LUMEN: GLOMERULAR ELIMINATION OF THE SAME SUBSTANCES[1]

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The behavior of the kidney toward certain injected dyes has long been used as an aid in the elucidation of renal function. Heidenhain's (1874) observation that the kidney was the specific excretory organ for indigocarmine led him to hope that intensive study of its behavior might help in the problem of the elimination of normal urinary constituents. Because of failure to see it in the glomerular structures and because of its obvious presence in the tubules, even of animals in which the kidney was eliminating no urine because of low blood pressure or ureteral obstruction, he assigned secretory power to the renal tubule and denied to the glomerulus the capacity of eliminating indigocarmine from the blood. Many investigators since have used a variety of dyes and of other substances susceptible of microscopic identification in the kidney substance in efforts to define and to localize the functions of its various parts. Unanimity of opinion concerning the elimination of these substances by the tubule has not been reached.

Two questions, intimately concerned with this type of study, have become approachable by the direct methods which have been recently introduced for the observation and instrumental manipulation of the frog's kidney:

1. Can the deposition of microscopically identifiable substances (dyes, iron, urea) within the cells of the renal tubule which occurs after intravenous or subcutaneous injection be duplicated in experiments in which the same substances are directly introduced into the lumen of the tubule.

2. Are the same substances when introduced into the blood stream eliminated from the blood in the glomerulus.

Affirmative answers to these two questions would permit the decision that glomerular elimination followed by tubular reabsorption or imbibition

[1] The results of some of these experiments were presented before the thirty-seventh annual meeting of the American Physiological Society, this Journal, lxxii, 184, 1925.

would be adequate to explain the results of many experiments which in the past have been interpreted as evidence of secretion by the tubule. The experiments described in this paper were designed to answer these two questions and the answer to both has been found to be affirmative.

I. INJECTION OF DYES, IRON SALTS AND UREA INTO THE TUBULE. *Methods.* Frogs (R. pipiens) were used in all experiments.

The substances studied were indigocarmine, sodium carminate, methylene blue, toluidin blue, trypan blue, phenolsulphonephthalein, ferric ammonium citrate and urea.

Two groups of experiments were made with each substance: in one group a minute amount of a solution of the substance was injected directly into the intracapsular space, from which it flowed into the tubule, by means of a capillary pipette, the point of which was thrust through the capsule of Bowman. The portion of the kidney which contained this single injected or irrigated tubule was excised, fixed and embedded in paraffin. The amount of fluid required for even a prolonged irrigation of a single tubule is so small (less than 0.005 cc.) that staining of the tubule as the result of absorption of the injected dye into the blood stream does not occur.

In the other group, which served as controls, a solution of the same substance was injected intravenously or into the ventral lymph sac, the animal killed after a suitable time, the kidneys excised, hardened and embedded in paraffin. Careful microscopic study of sections of the kidneys from the two groups enabled comparison of the deposition of the substance in the kidney resulting from each mode of access.

For making intracapsular (intratubular) injections, a technique similar to that devised by Wearn and Richards (1924) for collecting glomerular fluid was employed. The frog was pithed and the right kidney exposed for direct observation by transmitted light with a binocular microscope. The pipette was identical with that of Wearn and Richards and consisted of a quartz tip sealed into the long end of a glass 3-way stopcock tube, the opposite end of which was closed by a short piece of rubber tubing and glass rod, the perpendicular arm being connected with a levelling bulb. The whole apparatus was filled with mercury, a minute amount of the solution of the substance to be injected was drawn into the quartz tip, and the outside of the tip rinsed with distilled water.

After introducing the point of the pipette into the capsular space, a minute amount of the solution—considerably less than 0.005 cc.—was injected gradually or intermittently. The injection pressure distended the capsule and slightly compressed the glomerular tuft but did not stop flow of blood through it. The time of injection in different experiments varied from 20 seconds to 90 minutes. During the injection of the solution into the tubule no leakage occurred into surrounding tissue. At the end

of injection the pipette was withdrawn and the part of the kidney containing the injected tubule was cut out with the aid of the lowest power binocular objective.

Fixation methods, excepting in the experiments with phenol red, were chosen from the literature which we cite. Serial sections cut were from 6 to 10$\mu$ in thickness. Usually they were not counterstained: in some instances they were counterstained with picric acid, alcoholic fuchsin, eosin, or light green.

The results obtained with the individual substances studied will be outlined separately.

*1. Indigocarmine.* This dye, first used intravitally by Chrzonszczewsky (1864), chosen by Heidenhain for his classical work, is peculiarly unsuited for such experiments as we are describing. We worked extensively with it, however, because of its historical importance. It is unsuitable for several reasons: the tinctorial power of its solutions is not great; it is readily reduced to a leuco compound in living tissue; the diffuse and nuclear staining which is produced by it is now commonly believed to represent staining the cells only after their death. Furthermore, it is difficult to produce any staining of the amphibian kidney by injecting indigocarmine (Nussbaum, 1878; Gurwitsch, 1902; Kabrhel, 1886), though Basler (1906) believed that isolated granules within the cytoplasm are commonly stained in cold blooded animals.

In our control experiments in which Grübler's indigocarmine was injected intravenously or subcutaneously into frogs, we were never able to convince ourselves of staining of cytoplasm in the absence of nuclear staining. The amounts injected ranged from 0.25 to 4.0 cc. of 0.25 per cent solution; the frogs were killed at intervals ranging from 1 minute to 24 hours after the injection. The excised kidneys were at once placed in 50 volumes of absolute alcohol. Most of the sections were not counterstained: a few were counterstained with picric acid.

When 1 cc. or less of the solution was injected intravenously into a 50-gram frog, sections showed the presence of masses of the dye, both amorphous and crystalline, in the lumen of the tubules, no trace of color in the epithelial cells, and no color in the glomeruli. When larger amounts were used most of the cells of the convoluted tubules showed faintly blue nuclei and a barely perceptible blue tinge in the cytoplasm (plate I, *1*). In a kidney excised immediately at the end of a large injection some of the glomerular tufts showed a blue line around their free border, but no dye was seen within the capsular space. Staining of the cells of the tubules was more easily accomplished by perfusing the renal vessels with a solution of the dye in Ringer's fluid. Dead kidneys are readily stained in this way, the nuclei of the epithelium always being more deeply tinted than the cytoplasm.

In the experiments in which indigocarmine was introduced directly into a tubule of the living frog's kidney via the intracapsular space, the injection was made discontinuously. The first injection could be followed with the eye for only a short distance down the tubule, for the color quickly disappeared. After each succeeding injection a little more of the tubule became distinctly blue in contrast with those adjacent. The entire tubule did not appear uniformly blue: single loops and short sections were more intensely blue than intervening parts. After some ten minutes of such injections, particles of precipitated dye could be seen here and there in the stained sections of the tubule: and these particles could be driven down the entire length of the tubule and out of the field by slightly raising the mercury bulb. In this way one could see that all of the loops which he had been observing, whether deeply or faintly colored, were parts of one continuous tube.

Serial sections of that portion of the kidney which contained the injected tubule showed masses of dye in the lumen of the tubule: many, though by no means all of the nuclei were blue; the cytoplasm of some cells showed a faint bluish tinge (plate I, *2*).

Question may be raised whether the staining described is not the result of injury to cells by the strong solution of dye used (0.25 per cent). Weaker solutions were not easily visible as blue under the conditions of illumination used. The injected dye was diluted to an unknown extent by the glomerular fluid already in the capsule and by that which was eliminated in the glomerulus during and immediately after the injection. The appearance of precipitated dye indicates concentration in the tubule to saturation,

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Plate I. Camera lucida drawings of sections of frogs' kidneys (× 600).

*1.* Experiment 72 a. July 2, 1924. Kidney excised 30 minutes after intravenous injection of 4 cc. of 0.25 per cent indigocarmine at rate of 0.2 cc. every five minutes.

*2.* Experiment 40. March 17, 1924. Intermittent intracapsular injection of minute amount of 0.25 per cent indigocarmine for 45 minutes.

*3.* Experiment 51. April 15, 1924. Kidney excised 24 hours after injection of 1 cc. of a 1 per cent solution sodium carminate into the ventral lymph sac.

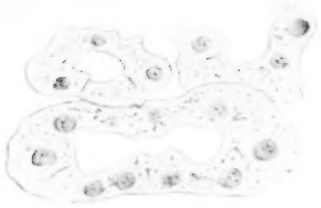
*4.* Experiment 81. July 17, 1924. Intermittent intracapsular injection of minute amount of 0.5 per cent sodium carminate for 20 minutes. Kidney removed one hour after circulation stopped.

*5.* Experiment 37. March 11, 1924. Kidney excised 21 hours after injection of 1 cc. of a saturated solution of toluidin blue into the ventral lymph sac.

*6.* Experiment 43. March 19, 1924. Intracapsular injection of a minute amount of half-saturated solution of toluidin blue for few minutes. Kidney removed one hour after circulation stopped.

*7.* Experiment A 2. January 31, 1925. Kidney excised 3 hours after injection of 1 gram of urea into the ventral lymph sac.

*8.* Experiment A 20. March 26, 1925. Intermittent intracapsular injection of a minute amount of 0.5 per cent solution of urea for one hour.



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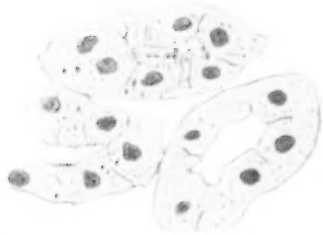

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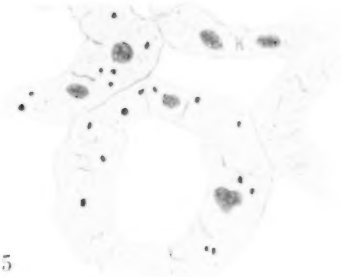

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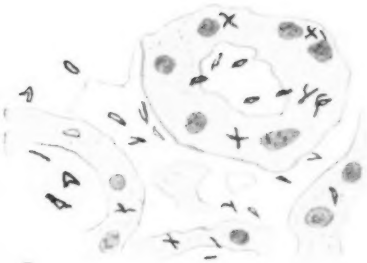

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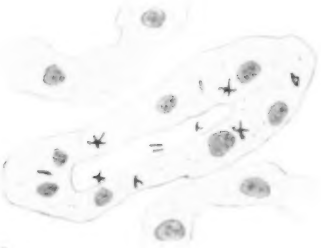

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and such concentration doubtless occurs in many tubules of animals injected with the dye intravenously. There is reason for thinking therefore that if this objection is valid for experiments on intracapsular injection it may be equally valid for experiments in which the dye reaches the kidney in the blood stream. The same statement may be made concerning the probability that the observed staining is due to post-mortem changes.

Protocols of two typical experiments follow.

*Intravenous injection.* July 2, 1924. *R. pipiens*, ♀, 40 grams. Brain pithed; ovaries excised; cannula in anterior abdominal vein; 4 cc. 0.25 per cent Grübler's indigocarmine injected at rate of 0.2 cc. every 5 minutes. Skin, muscles, liver and kidneys intensely blue. Bladder filled with blue urine. Kidneys removed 30 minutes after end of injection; in absolute alcohol 24 hours; embedded in paraffin. Section, 10$\mu$. Some counterstained with picric acid. Amorphous and crystalline masses of dye in lumen of many tubules. A few glomeruli were outlined in blue. Most of the cells of the convoluted tubules show faintly blue nuclei with more deeply stained granules in them. Cytoplasm of cells is a scarcely perceptible blue.

*Intracapsular injection.* March 13, 1924. *R. pipiens*, ♀, 40 grams. Brain pithed ovaries excised, right kidney prepared for transillumination. Capillary pipette, containing 0.25 per cent Grübler's indigocarmine, introduced through capsule of Bowman into capsular space without difficulty. Injection discontinuous.

After 5 minutes some loops of the tubule were visibly blue: after 7 minutes blue granules could be seen in some. Injection continued intermittently for 30 to 40 minutes. Pipette withdrawn. The part of the kidney which contained this tubule was cut out, fixed in absolute alcohol and serial sections cut from paraffin. Masses of dye in lumen of tubule. Many nuclei of cells are deep blue. Cytoplasm of cells which show nuclear stain is faintly blue. Sections of tubules at a distance from the injected tubule show no stain.

*2. Carmine.* After injection of carmine or one of its salts, the dye is usually found in the glomeruli and capsules as well as in the lumen and cells of the tubules. Heidenhain's followers believed that this evidence of glomerular elimination indicated injury to the capillaries (Schmidt, 1891; Bilerfeld, 1904, 1907; Basler, 1906) but others obtained it in experiments in which injury was unlikely (Sobieranski, 1895; Arnold, 1902; Schlecht, 1906). In the tubule cells the dye is deposited on the intracellular granules. Staining of cells is first detectable about 15 minutes after the dye is injected, and appears close to the brush border of the proximal convoluted tubules (Mitamura, 1924); later the granules increase in size and number, and are found nearer the basement membrane. This granular staining is present for days after the carmine is no longer detectable in the urine, and consequently cannot be regarded as evidence of secretion of the dye (Suzuki, 1912). Mitamura showed, as had Basler, that the dye may be present in the urine before any deposit is detectable within the tubule cells. Aschoff (1924), in whose laboratory Mitamura worked, accepted his results as evidence of reabsorption rather than of secretion. v. Möllendorf (1920) has expressed the same opinion.

In our control experiments, 1.0 to 1.5 cc. of 1 per cent sodium carminate solution (Grübler) was injected into the ventral lymph sac. Twenty-four hours later the animals were killed, the kidneys hardened in absolute alcohol and sections cut from paraffin. The cells of many convoluted tubules showed an abundance of red granules, most of which were grouped around the nucleus (plate I, *3*): many sections of the convoluted tubules contained no dye and no staining of structures in the Malpighian body was apparent.

In experiments in which the dye was injected into the capsular space, a 0.5 per cent solution was used. Precipitation of the dye could be observed within the lumen of the tubule as has been described for indigo-carmine. Circulation through the kidney was stopped at the end of the injection and some minutes allowed to elapse before transferring the piece of kidney containing the stained tubule into absolute alcohol.

Examination of the sections showed masses of dye within the tubule, often surrounded by unstained cells. At other levels the tubule cells contained red granules in all the positions seen in the control experiments (plate I, *4*). The dye was often found in the brush border and along the free margin of the cell. In other instances the cytoplasm of the cell appeared diffusely red from lumen border to nucleus, due to the presence of innumerable minute red granules. The size of the stained granules in the cells of the kidneys of frogs injected subcutaneously was as a rule greater than was the case when the dye was introduced directly into the tubule—a fact possibly due to difference in time of contact.

With the exception of an occasional stained nucleus in the intracapsular injection experiments, the greater masses of dye in the lumen, and the scarcity of large stained granules, the appearance of the control kidneys and those reached by intracapsular injection was the same.

Protocols of two typical experiments follow.

*Subcutaneous injection.* April 15, 1924. *R. pipiens*, ♀, about 50 grams. Injected with 1.0 cc. of a 1 per cent solution of Grübler's sodium carminate into the ventral lymph sac. Killed 24 hours later. Kidneys pink. In absolute alcohol 24 hours; embedded in paraffin. Sections counterstained with light green. No stained glomeruli: no dye in lumen of convoluted tubules. Cells of convoluted tubules in many sections showed abundance of red granules in cytoplasm in all positions, possibly being most numerous near the nucleus. Other sections showed no staining.

*Intracapsular injection.* April 10, 1924, *R. pipiens*, ♀, about 40 grams. Brain pithed; ovaries removed; right kidney prepared for transillumination. Capillary pipette containing 0.5 per cent sodium carminate introduced into capsular space of glomerulus showing very active circulation. Injection continued for 45 minutes. After the injection had continued for some minutes the dye could be seen to be aggregated into minute particles in the lumen of the tubule. Kidney excised 20 minutes after end of injection, circulation having been stopped during that time. Absolute alcohol 24 hours; serial sections from paraffin; light green counterstain. Dye

is found as amorphous masses in lumen, the adjacent cells often being colorless. Many other cells contain minute or larger granules in all positions, but as a rule more between the nucleus and lumen border.

*3. Methylene blue.* Gurwitsch (1902) has pictured the diffuse distribution of granules in the cells of the tubules of kidneys of frogs which had received methylene blue by mouth and expressed the view that they represent secretory activity. Arnold (1902) injected mice subcutaneously and pictures blue granules first near the lumen border, later widely distributed through the cells. Höber and Königsberg (1905) described the deposition of methylene blue as a fine dark blue dust in the cells and were emphatically of the opinion that the cells of the tubules eliminate it.

In our experiments, a 1 per cent solution of Grübler's methylene blue in 0.6 per cent NaCl was used both for injection into the ventral lymph sac in the control experiments and into Bowman's capsule for the intracapsular injection experiments. The dye was fixed by immersion in 5 per cent ammonium molybdate and sections were cut from paraffin. The mode of deposition of the dye within the tubules is described in the following two protocols, which are completely typical.

*Subcutaneous injection.* March 29, 1924. *R. pipiens*, ♀, about 50 grams; 1.5 cc. of 1 per cent methylene blue injected into ventral lymph sac. Killed 2 hours later, having passed blue urine. Kidneys blue. In 5 per cent ammonium molybdate 5 hours. Hardened in alcohol. Sections cut 10$\mu$. Small amounts of the dye in lumen of the convoluted tubules, generally adherent to the border of the cells. Cytoplasm of about half the cells contains very minute blue granules, giving appearance of diffuse blue stain. Membrana propria deeply stained. In places the dye seems to extend from the ring of stain in the lumen in streaks between the cells (plate II, *1*).

*Intracapsular injection.* March 10, 1924. *R. pipiens*, ♀, about 50 grams. Brain pithed; ovaries excised; right kidney prepared for transillumination. Capillary pipette, containing 1 per cent methylene blue in 0.6 per cent NaCl, introduced through the capsule of a glomerulus in which blood flow was very active. Immediately after beginning injection the capsule and many loops of convoluted tubule became intensely blue. Within half a minute, narrower loops (distal convolutions) became blue. No visible precipitation of dye occurred. On interrupting the injection the color faded. The injection was continued for 10 minutes, and the circulation stopped 20 minutes before cutting out the piece of kidney for fixation. Serial sections, 10$\mu$ thick, were cut from paraffin. Dye in the lumen of the tubules adheres to the cells. Very minute blue granules are seen throughout the cells. The basement membrane is stained and dye can be seen in the peritubular spaces. Streaks of dye between the cells seem to connect the stained basement membrane with the dye within the lumen. An occasional nucleus is stained (plate II, *2*).

*4. Toluidin blue.* The staining qualities of the renal epithelium as revealed by the introduction of this lipoid-soluble dye formed the chief basis of the conceptions developed by Gurwitsch (1902) concerning the part played by intracellular vacuoles in the excretory function of the tubules. He pictured the appearance of blue granules in various positions and of various

sizes and regarded them as representing different stages in the progress of the dye through the tubule cells from base to free border. Höber and Königsberg (1905) amplified these observations and convinced themselves of the correctness of the general conceptions.

Our observations were made with toluidin blue prepared by the Harmer laboratory. Two typical protocols show the essential similarity of the staining picture obtained from subcutaneous injection with that produced by direct introduction into the lumen of the tubule.

*Subcutaneous injection.* March 11, 1924. *R. pipiens*, ♂, about 40 grams; 1 cc. of saturated solution of toluidin blue injected into the ventral lymph sac. Killed after 4 hours. Skin and fascia blue: muscles not stained; kidneys reddish blue. Kidneys in saturated $HgCl_2$ 1 hour; saturated NaCl, ½ hour; potassium bichromate over night; 4 per cent ammonium molybdate, 6 hours; dehydrated in alcohol, cut from paraffin, some sections counterstained with picric acid. Blue masses in lumen of tubules, in peritubular spaces and in the blood vessels. Bluish purple granules in cells of convoluted tubules between nucleus and lumen border, also between nucleus and basement membrane. Nuclei unstained. Some granules are so small as to be barely visible; others are a quarter as large as the nucleus (plate I, *5*).

*Intracapsular injection.* March 19, 1924. *R. pipiens*, ♀, about 50 grams. Brain pithed, ovaries excised, right kidney prepared for direct observation. Good circulation. Capillary pipette, filled with half-saturated solution of toluidin blue in 0.6 per cent NaCl, inserted into a Malpighian body very close to surface and near edge of kidney. Circulation in glomerulus slower after insertion. Duration of injection, ten minutes. No granules of precipitated dye could be seen in the lumen. Pipette withdrawn; flow in tuft slow. Watching for half an hour, the color seemed to fade slightly. Circulation stopped and kidney fraction excised half hour later.

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Plate II. Photomicrographs of sections of frogs kidneys. Appropriate Wratten filters were used to intensify the contrast between deposited dye and unstained tissue.

*1.* Experiment 45. March 29, 1924. Killed 2 hours after injection of 1.5 cc. of a 1 per cent solution of methylene blue into ventral lymph sac (× 400).

*2.* Experiment 44. March 19, 1924. Kidney removed 20 minutes after intermittent intracapsular injection of a minute amount of 1 per cent methylene blue solution lasting 10 minutes (× 400).

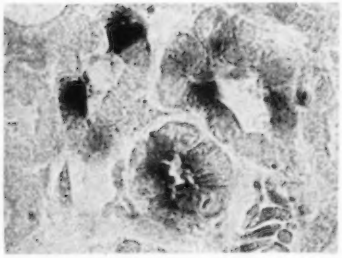
*3.* Experiment 80. July 10, 1924. Two injections, each of 2 cc. of 0.1 per cent trypan blue solution, into the ventral lymph sac. Killed and kidney excised 24 hours after the first, 3 hours after the second injection (× 700).

*4.* Experiment 82. July 17, 1924. After intermittent intracapsular injection of a minute amount of 0.05 per cent solution of trypan blue for 90 minutes (× 700).

*5.* Experiment 63. June 2, 1924. Killed 1 minute after the end of an intravenous injection of 2.5 mgm. each of potassium ferrocyanide and ferric ammonium citrate (× 700).

*6.* Experiment 59. May 22, 1924. Kidney excised immediately after intracapsular injection of a minute amount of 1 per cent potassium ferrocyanide and ferric ammonium citrate for 20 seconds (× 700).

*7.* Experiment 54. May 8, 1924. Kidney excised immediately after an intracapsular injection of a minute amount of 0.6 per cent phenolsulphonephthalein lasting about 2 seconds (× about 100).



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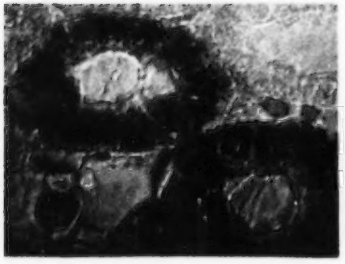

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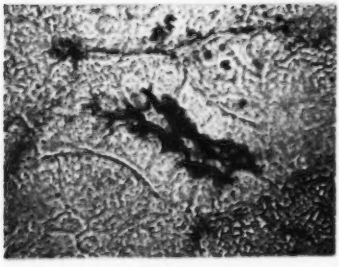

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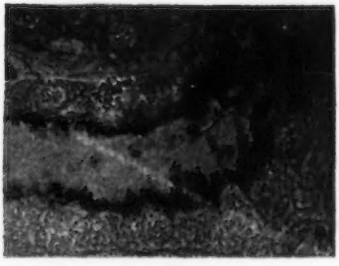

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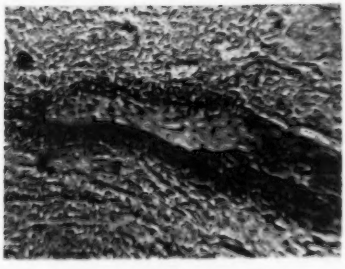

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Fixation as described above. Precipitated dye in the lumen of tubule; cells of convoluted portion contain blue granules of various sizes in all positions (plate I, *6*).

*5. Trypan blue.* Goldman (1909) described staining reactions of the mammalian kidney with trypan blue which led him to accept for this substance a conception of its secretion by the tubules similar to that developed by Gurwitsch for the secretion of methylene blue and toluidin blue. v. Möllendorff on the other hand states that the first detectable deposition of the dye in the convoluted tubules of mice occurs in the half of the cell nearer the lumen. Peter (1924), working with larvae of *Salamandra maculosa* and *Triton taeniatus*, observed accumulation within the tubule cells close to the free border, granules near the basement membrane appearing only later; and similar observations in a variety of mammals and in frogs were made by de Haan and Bakker (1923).

We have used typan blue prepared in the chemical laboratories of the Phipps Institute of this University. In frogs which received the dye by subcutaneous injection and were killed twenty-four hours later, little dye was found in the lumen of tubules but in some sections the cells of the proximal convoluted tubules contained an abundance of blue granules: in some instances they were very minute and were distributed uniformly throughout the cell; in others they were massed into clumps half as large as the nucleus and were situated near it (plate II, *3*). In a single slide striking contrast was to be seen between sections of tubules quite unstained and those heavily stained, only few showing intermediate intensity of staining.

In experiments in which the dye was introduced directly into the lumen of the tubule via the intracapsular space its progress down the tubule could be followed with the eye only with difficulty; but after some minutes a loop of the proximal tubule at some distance from the punctured capsule began to stand out as faintly blue. The injection continuing, color less intense extended further along this tubule in either direction from the loop first stained. The impression was gained that the staining is highly selective for a particular part of the tubule, though it is impossible to state the exact relation of this to the glomerulus because of the impossibility of identifying the entire length of the injected tubule. In sections of the portion of the kidney containing this tubule, cells containing abundant blue granules in all positions were found in a part of the tubule which was in the same position relative to the glomerulus as was the loop of tubule seen to be deeply colored during life. The pictures of these sections of the tubule differed from those obtained after subcutaneous injection only by the greater amount of dye in the lumen of the tubule (plate II, *4*).

Protocols of two typical experiments follow:

*Subcutaneous injection.* July 10, 1924. *R. pipiens*, ♀, about 40 grams; 2 cc. 0.1 per cent trypan blue injected into ventral lymph sac at 4:00 p.m. This dose

repeated on the following day at 2:00 p.m. and the frog was killed three hours later. Skin, muscles, fascia and intestines blue; liver purple; kidneys deep blue. Kidneys in 10 per cent formalin over night, then transferred to alcohol. Sections 7$\mu$ in thickness, counterstained with eosin. Very little dye in the lumen of convoluted tubules. Some sections, apparently of proximal convoluted tubules, show epithelial cells containing many large blue granules. Other sections of the tubules contained no dye.

*Intracapsular injection.* July 17, 1924. *R. pipiens*, ♀, about 40 grams. Brain pithed; ovaries excised; right kidney prepared for direct observation. Capillary pipette, containing 0.05 per cent trypan blue in 0.6 per cent NaCl, introduced into intracapsular space. Intermittent injections over 90 minutes. Total volume of fluid injected not more than 0.001 cc. At first the fluid could not be seen in the tubule. After injecting enough to fill the capsule of Bowman every one or two minutes for 15 minutes, an S-shaped loop of tubule began to stand out as faintly blue. The intensity of color in this loop increased as injections were repeated, while other loops apparently distal to this became clearly visible, though less intensely stained. When no injection was made for some minutes, these latter slowly faded while the stain in the first loop persisted unchanged. The tissue was fixed in formalin followed by alcohol. Sections, 7$\mu$ thick, showed masses of dye in the lumen and in a few sections the epithelial cells contained numerous blue granules, chiefly between nucleus and lumen.

6. *Phenolsulphonephthalein.* Accurate description of the deposition of this dye within the kidney is extremely difficult because of its ready diffusibility and because it is soluble in all of the ordinary reagents used for fixing and hardening tissues for microscopic study. Recent evidence, experimental rather than histological, has been advanced by Marshall and his colleagues and by Starling and Verney (1925) in support of the view that the tubules take active part in the elimination of phenol red. Marshall and Vickers (1923), having discovered a high degree of concentration of the substance in the renal cortex of dogs, examined fresh sections with the microscope and reported the presence of the dye in the cells and not in the lumen. (The spinal cord of the dog from which this kidney was taken had been cut before injection of the dye.) Bieter and Hirschfelder (1924) observed the ventral surface of the living frog's kidney during and after injections of phenol red and saw no evidence of accumulation in tubule cells. Edwards and Marshall (1924), however, state that when the dorsal surface is watched the walls of the tubules take on color indicative of accumulation in cells. They also observed the surface of the rat's kidney and believe that similar accumulation in cells of the proximal convolutions occurred in those animals. They concede that there are pitfalls in this type of study.

Our efforts were first devoted to the discovery of a method by which phenol red could be fixed *in situ* and its distribution studied in paraffin sections. The dye is insoluble in xylol, chloroform and absolute ether. We found that fairly adequate dehydration and hardening of the kidneys

was accomplished by perfusing about 15 cc. of dry ether through the renal arteries. They were then excised, placed successively in absolute ether (twenty-four hours), chloroform (four hours), chloroform and paraffin (four hours), and finally embedded in paraffin. Sections were transferred directly from the microtome knife to the slide (floating on water dissolves the dye), mounted in xylol saturated with ammonia gas, and ringed with wax. The tissue is shrunken and brittle, but its structure is sufficiently preserved to permit identification of cell nuclei and cytoplasm, glomeruli and lumen of tubules. The color in such slides fades after twenty-four to forty-eight hours.

In sections of kidneys taken from frogs which had been injected intravenously with phenol red the cytoplasm of the cells of all sections of the tubule was diffusely pink, the nuclei being paler or unstained.

Introduction of a solution of the dye directly into the lumen of the tubule produced effects quite different from those observed during the injection of other dyes. With the latter, the color was always confined definitely to the single tubule in which injection was made—a fact which in most cases permitted the certain and easy identification of its convolutions. After the injection of phenol red had continued for five minutes an increasing, ill-defined area of color could be seen extending across the field from the injected capsule. In this area loops of the injected tubule stood out as a result of their slightly more intense color.

After the intracapsular injection had been finished, the minute piece of kidney containing the colored area was at once dropped into a large volume of absolute ether, subsequently treated with chloroform, and embedded in paraffin as has been described.

In experiments in which 0.002 cc. or more of the phenol red solution had been injected into the tubule, the sections showed a V-shaped area in which all visible structures were stained uniformly pink. More satisfactory results were obtained by injecting a much more minute amount of the solution, immediately withdrawing the pipette and cutting out the piece of kidney. In such a preparation a loop of tubule at some distance from the glomerulus showed a pink stain in the epithelial cells which was more intense at the lumen border and faded gradually toward the basement membrane. (A low-power photomicrograph showing such a tubule is reproduced in plate II, 7.)

Two typical protocols follow:

*Intravenous injection.* May 15, 1924. *R. pipiens*, ♀, about 50 grams. Brain pithed; ovaries excised; cannula in anterior abdominal vein; 9 mgm. of phenolsulphonephthalein injected during 10 minutes. During the following 45 minutes 0.25 cc. of red urine was eliminated. At the end of this time, a cannula was inserted into the central stump of the coeliaco-mesenteric artery, the aorta tied above this and below the kidney, and 12 cc. of dry ether perfused through the renal vessels. The

last 2 cc. of this were quite clear as it issued from the renal vein. Sections (10$\mu$) were prepared as described above. The tubular epithelium was stained a uniform diffuse pink, nuclei paler than cytoplasm. The glomeruli and capsules were also pink.

*Intracapsular injection.* May 8, 1924. *R. pipiens*, ♀, about 40 grams. Brain pithed; ovaries excised; right kidney prepared for transillumination. Capillary pipette containing 0.6 per cent phenol red in 0.6 per cent NaCl was introduced into an intracapsular space. The dye was run in at less than diastolic pressure for about 2 seconds until a few loops of tubule could just be seen. Pipette withdrawn, heart excised and the piece of kidney (2 × 3 mm.) cut out and put in absolute ether as rapidly as possible. Serial sections were cut 8$\mu$ thick.

Extending inward from the edge of the kidney is a broad V-shaped area in which all structures are uniformly pink. This area obviously includes more tubules than the one injected. It represents in section the ill-defined areas of color which were seen during more prolonged injection in other frogs. Cross sections of a tubule can be identified in several sections outside of this area, separated from it by unstained tissue, in which the cells are stained, the color fading toward the basement membrane. Visible nuclei are not stained more intensely than cytoplasm.

7. *Ferric ammonium citrate.* Microchemical identification of iron in the cells of the convoluted tubules of dogs and rabbits, following the injection of soluble double salts of iron, was made by Kobert (1883) and by Glaevecke (1883). Each decided that it was secreted by these cells, though the latter describes its position in the cells as close to the free border. Confirmatory observations are to be found in the work of Biberfeld and of Basler. More detailed study was made by Stieglitz (1921). Kidneys taken from rabbits two to three minutes after intravenous injection of ferric ammonium citrate showed granules of iron in all positions in the cells of the convoluted tubules; when ten minutes or more had elapsed, the accumulation was discerned near the lumen border. These observations are interpreted as evidence of secretion of iron into the tubule. Dawson (1925) injected iron salts into the body cavity of necturus and found microchemical evidence of iron in the cells of primary tubules having peritoneal openings before sufficient time had elapsed for absorption into the blood stream. After intravenous injections he found iron in the endothelium and epithelium of the glomerulus, in the lumen of the ciliated neck of the tubule and in the cells close to the lumen border.

We have used a slight modification of the methods of Stieglitz. A slightly alkaline solution of green ferric ammonium citrate and potassium ferrocyanide was injected, in one series of experiments intravenously, and in the other directly into the tubule. The iron in the kidney was precipitated as prussian blue by trichloracetic acid. Both series showed blue granules within the cells, chiefly between the nuclei and the lumen border, with a few occasional granules nearer the basement membrane (plate II, *5* and *6*).

*Intravenous injection.* June 2, 1924. *R. pipiens*, ♀, about 40 grams. Brain pithed; ovaries excised; cannula in anterior abdominal vein; 0.25 cc. of a solution containing green ferric ammonium citrate and potassium ferrocyanide (each 1.0 per cent), 0.6 per cent NaCl, and $Na_2CO_3$ 0.25 per cent, injected during three minutes. The frog was killed one minute after the end of the injection and 5 per cent trichloracetic acid at once perfused through the renal arteries via the aorta. The excised kidneys were placed in trichloracetic acid for one hour, absolute alcohol over night, then passed through chloroform and paraffin. Sections, 6$\mu$, counterstrained with eosin.

Blue granules found in the lumina of tubules, chiefly adherent to the cell borders. Other granules are inside the cells between the nucleus and free border, apparently continuous with granules caught in the ciliated border. In some instances the blue extends from lumen toward basement membrane apparently between the cells. A few granules within the cells are situated about the nuclei and near the basement membrane.

*Intracapsular injection.* May 5, 1924. *R. pipiens*, ♀, about 50 grams. Brain pithed; ovaries excised; right kidney prepared for direct observation. Capillary pipette containing the solution of ferric ammonium citrate and potassium ferrocyanide described above introduced through the capsule of an actively flowing glomerulus. Injection continued for 20 seconds; pipette withdrawn, a drop of 5 per cent trichloracetic acid placed on the surface of the kidney. The circulation was stopped the portion of the kidney containing the injection tubule excised and passed through trichloracetic acid, alcohol, chloroform and paraffin. Serial sections, 6$\mu$. A blue precipitate is adherent to the lumen border of the cells. There are granules within the cells chiefly between the nucleus and the lumen, some, however, between the nuclei and basement membrane. In some sections the blue mass within the lumen appears to extend between the cells toward the basement membrane.

8. *Urea.* The microchemical identification of urea within the cells of the convoluted tubules with the aid of the xanthydrol reaction has been regarded as evidence of secretion of urea by these cells by Chevallier and Chabanier (1915), Oliver (1921), Stübel (1921), Walter (1923) and others. When a solution of xanthydrol is perfused through the renal vessels crystals of the di-xanthydrol compound are precipitated in the tissue and may be identified by their form and by their behavior toward polarized light.

We have followed the method of Chevallier and Chabanier, using a saturated solution of xanthydrol in equal parts of ether and glacial acetic acid for perfusion of the renal arteries.

The normal urea content of the kidneys of fasting frogs is apparently insufficient to permit microscopic identification. When urea was injected into a lymph sac one or two hours before preparing the kidneys, characteristic crystals were demonstrable in all parts of the kidney, especially abundant in the cells of the convoluted tubules (plate I, 7). Similar amount and distribution of crystals were found in experiments in which minute amounts of urea had been injected into the kidney via the ureter, and also in dead kidneys which had been immersed in a urea solution before perfusion with the xanthydrol reagent.

In the experiments in which urea was injected directly into the lumen

of the tubule it was necessary to add to the injection fluid another substance the presence of which should make certain the identification in sections of the tubule which had been injected *intra vitam*. For this purpose a mixture of ferric ammonium citrate and potassium ferrocyanide was used, the acid of the xanthydrol reagent serving to precipitate the prussian blue.

Sections of the tubule which had been injected with urea solution showed abundant crystals of the xanthydrol urea compound in the lumen of the proximal convolutions, within the epithelial cells, and in the peritubular lymphatics (plate I, *8*). Some crystals were also found in the cells of tubules whose lumina contained no prussian blue, adjacent to sections of tubules which did. Sections of tubules at a distance from those which contained prussian blue did not contain urea crystals.

*Subcutaneous injection.* January 31, 1925. *R. pipiens*, ♀, about 40 grams. One gram of urea in 1 cc. water injected into ventral lymph sac. One hour later the brain was pithed, ovaries excised and a cannula inserted into the central stump of the coeliaco-mesenteric artery. The renal vessels were perfused first with 0.2 to 0.3 cc. of 95 per cent alcohol, then with 2 cc. of the xanthydrol reagent. The excised kidneys were immersed in the xanthydrol reagent for 24 hours. Paraffin sections, 9$\mu$; some stained with hematoxylin.

In the sections, crystals of dixanthydrol urea are abundant in blood vessels, glomerular capillaries, Bowman's space, peritubular lymphatics and tubule cells.

*Intracapsular injection.* March 25, 1925. *R. pipiens*, ♀, about 50 grams. Brain pithed; ovaries excised; right kidney prepared for direct observation. Cannula in proximal stump of aorta below kidneys for subsequent perfusion with reagent: loose ligature about aorta above renal arteries. Capillary pipette containing urea, ferric ammonium citrate, and potassium ferrocyanide (each in 0.5 per cent concentration), dissolved in 0.6 per cent NaCl, was introduced into the capsular space of an active glomerulus. Intermittent injections continued for one and a half hours. The aortic ligature was tied immediately after final discontinuance of injection and the heart excised. Two-tenths cubic centimeter of 95 per cent alcohol was perfused through the renal arteries followed by 2 cc. of the xanthydrol reagent. The piece of kidney containing the injected tubule was treated as described above. Serial sections, 9$\mu$, cut from paraffin. The prussian blue precipitate is found most abundantly in the lumen of the tubule; some granules are within the cells of the convoluted portion and in peritubular spaces. Crystals of the urea compound are found in the lumen along with the blue precipitate, in the cells of this tubule and in peritubular spaces. There are a few crystals in cells of tubules which do not contain prussian blue. No crystals in sections remote from the injected tubule.

II. GLOMERULAR ELIMINATION OF INDIGOCARMINE, SODIUM CARMINATE, METHYLENE BLUE, TOLUIDIN BLUE, TRYPAN BLUE, PHENOLSULPHONEPHTHALEIN, IRON SALTS AND UREA. In the experiments thus far outlined indubitable evidence has been secured that when the lumen of the frog's renal tubule is directly injected or irrigated with a solution of any one of the substances named, the substance itself enters the cells lining the tubule in quantity sufficient to make microscopic identification in fixed sections easy. Under these conditions also the manner of distribution of the

substance within the cells is indistinguishable from that which is discovered in experiments in which the substance has reached the kidney by the blood stream following intravenous or subcutaneous injection.

In order to show however that these observations are useful in interpretation of the results of histological study of kidneys intravitally injected in the usual manner, it is necessary to know whether or not all of the substances studied are eliminated from the circulation by the glomerulus.

For some we already have direct evidence. After intravenous injection apparent passage of indigocarmine, phenol red, and methylene blue from the glomerular vessels into the capsular space in frogs has been observed by Wearn and Richards (1924) and by Bieter and Hirshfelder (1924); of trypan blue by Hirshfelder and Bieter (1925). The presence of the first three dyes was demonstrated by Wearn and Richards in fluid collected directly from Bowman's capsule. Urea has been identified as a normal constituent of glomerular fluid in frogs, both by the xanthydrol test and by Nessler's reaction after hydrolysis. In view of the directness of this evidence it seems unnecessary to cite the older literature of debate on the glomerular elimination of these substances.

Experiments with all of the other substances, whose behavior toward the tubule has been described above have been made by the method of Wearn and Richards. In each instance the mercury-filled quartz capillary pipette was inserted through the capsule of Bowman into the capsular space of an active glomerulus of a pithed or anesthetized frog and collection of glomerular fluid begun. The substance to be studied was then injected by means of a cannula in the anterior abdominal vein and glomerular fluid collection continued until a sufficient amount for inspection or test had accumulated. The following protocols are illustrative.

*Sodium carminate.* June 3, 1924. *R. pipiens*, ♀; brain pithed. Cannula in anterior abdominal vein: 0.6 per cent NaCl injected intravenously at intervals during preparation—total, 1.0 cc. Glomerular circulation active. Glomerular fluid collection begun at 2:30. Shortly after, injected 1.5 cc. of 1 per cent sodium carminate solution into vein at rate of 0.2 cc. every 5 to 10 minutes. Collection of glomerular urine rapid: stopped at 3:30. Fluid discharged on a white clay surface left a red spot of about the same color intensity as that left by a similar amount of the bladder urine simultaneously collected.

*Ferric ammonium citrate.* May 20, 1924. *R. pipiens*, ♀; pithed. Cannula in anterior abdominal vein. One cubic centimeter 0.6 per cent NaCl injected during the preparation. Glomerular blood flow active. Collection of glomerular urine begun at 1:15. One cubic centimeter of a solution of green ferric ammonium citrate was injected by vein in five fractions five minutes apart. Pipette withdrawn at 2:15. Glomerular fluid discharged on a white paper previously treated with potassium ferrocyanide and trichloracetic acid left a deep blue spot of about the same color intensity as that left by a similar amount of bladder urine simultaneously collected. The color was deeper than that given by a sample of the original solution injected.

*Toluidin blue.* July 12, 1926. *R. pipiens*, ♂: about 20 grams. One-eighth gram urethane in ventral lymph sac. Subcutaneous injection 0.6 per cent NaCl during preparation. Fairly good circulation. Glomerular collection begun at 3:45. During the collection 1.5 cc. of a 0.5 per cent solution of toluidin blue was injected subcutaneously at rate of 0.2 cc. every 5 to 10 minutes. Could not see color in capsular space, but after about an hour tubules became outlined more distinctly and seemed to contain bluish black dots. Pipette withdrawn at 5:10. Fluid discharged on a white clay surface left a definite blue spot. Bladder urine collected simultaneously left a somewhat more intense stain.

*Trypan blue.* July 13, 1926. *R. pipiens*, ♂; about 25 grams; 0.2 gram urethane in ventral lymph sac. Cannula in anterior abdominal vein. One cubic centimeter of 0.6 per cent NaCl injected during preparation. Venous flow sluggish, but one active glomerulus in field with distended capsule. Pipette inserted at 10:30. During the collection injected 1.75 cc. of a 0.25 per cent solution of trypan blue intravenously at rate of 0.2 cc. every 5 to 10 minutes. Pipette withdrawn at 12:35. Whole frog quite blue. The small amount of fluid collected left a blue spot when discharged on a white clay surface. Bladder urine left a slightly deeper stain.

III. Incidental observations. Certain observations of interest were made during the injections: when the mercury leveling bulb was raised slightly above the level of the kidney the capsule distended and the capillary tuft shrunk: on turning the tap so that injection ceased, the capsule contracted and the tuft expanded. Flow of blood through the capillaries of the tuft became respectively slower and more rapid with these manipulations—illustrating the dependence of glomerular blood flow on intracapsular pressure. The exquisite sensitivity of the tuft to slight changes in intracapsular pressure, as exhibited by changes in size, was constantly used as a means of assuring ourselves of the proper insertion of the point of the pipette into the capsule.

When an injection was interrupted or discontinued, the colored fluid could be seen to be washed on down the tubule, obviously by fluid leaving the glomerular blood vessels. White and Schmitt (1925) have reported similar experiments with Necturus.

Evidence of reabsorption of fluid from the tubule is readily obtained in this type of experiment. Before the injected solution of indigocarmine or sodium carminate has progressed through many loops of the tubule a precipitate of the dye can be observed to have formed in the lumen of the tubule, a fact most readily explainable by reabsorption of water.

The complexity of the renal tubule in the frog is vividly revealed by this method of intra vitam injection. In figure 1 are reproduced rough sketches of the coils of single tubules made at the time of injection. (The injected tubule is difficult to photograph *in situ* because of the different planes in which its coils are arranged.) As many as seventeen cross sections of one injected tubule have been counted in a single section cut from a kidney injected in this manner. Possibly the method of direct intracapsular

injection could be extended with advantage to morphological studies of renal tubules.

Difficulties of identifying by direct inspection the exact location of a dye within the living kidney have been impressively demonstrated in these experiments. Repeatedly after the intravenous injection of indigocarmine we have felt sure that certain granules or accumulations of the dye seen during life were within the cells of the tubules; in cut sections, however, the chief accumulations of dye were adherent to the lumen border of the cells. When the more diffusible dyes are used, caution is necessary in designating the plane of observation as colored because of the possible presence of color in the planes above or below it. Irwin (1922) has emphasized the possibility of error in estimating the degree of color of cell contents because of adsorption of dye on cell wall or surface.

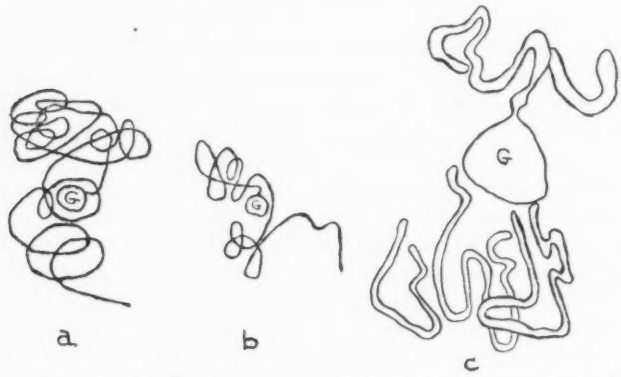


Fig. 1. Sketches of the convolutions of single tubules as identified by the intracapsular injection of *a*, indigocarmine; *b*, toluidin blue; *c*, methylene blue.

Discussion. Eight substances of widely different qualities have been studied in relation to their behavior in the kidneys of frogs. Among them are two or three substances (e.g., indigocarmine, iron) about which has centered much controversy concerning glomerular elimination and tubular secretion. After introduction into the blood, each has been found to be eliminated in the glomerulus; after direct introduction into the lumen of the tubule, each has been found to enter the cells of the tubule. Hence we have proof that in the frog deposition of any of these in the tubule cells after the usual modes of administration can be explained without the assumption of secretion by the tubules.

Failure to discover in glomerular structures in fixed kidney sections traces of a substance injected into the circulation is not now to be regarded

as competent evidence against glomerular elimination of that substance. The controversy on this point in connection with indigocarmine is well known: most of the older observers failed to find iron in the Malpighian body: and in our experience similar failures have been encountered. In every instance, however, the more valid tests of direct observation and examination of fluid drawn from the capsule of Bowman have yielded positive evidence of glomerular elimination. The degree of dilution of the substance in the glomerular fluid, the character of the lining epithelium of the capsule of Bowman in contrast to that of the tubule, and the mechanical ease of washing out the contents of the spaces in the kidney by fixing reagent are all to be thought of in this connection.

The character and distribution of the deposits of the substances which we have studied in the tubule cells by the two modes of introduction is so closely identical that the applicability of our results of intratubular injections to those of intravenous or subcutaneous injection seems assured: and the resemblance of our descriptions to those supplied by previous workers is sufficiently close to justify their application to those.

The objection may be raised that we have introduced into the capsule and hence into the tubule stronger solutions of the substances studied than are to be found there following intravenous injection. To this it may be answered that our solutions were diluted as they were introduced by the fluid present and being formed within the capsule: that reabsorption of water in the tubule is known to occur: that there is every assurance that the degree of water reabsorption is greater than that of most if not all of the substances we have used: hence that concentrations of injected substances approaching saturation may occur within the tubules even following the intravenous injection of dilute solutions. This is the reasoning used by Sobieranski who first effectually took issue with Heidenhain and obtained evidence from which our results might have been predicted.

Our results are not offered as further proof of the existence of processes of reabsorption in the tubules, though they harmonize with the known facts concerning this; nor are they offered as evidence against the existence of secretory processes in the renal tubule. In the former connection, they show only that between the time of introduction into the tubule and that of examination of the sections some of the injected substance has found its way into the tubule cells. That the activity of the cell plays a rôle in this phenomenon has not been shown. It is possible that post-mortem diffusion, imbibition, or adsorption take part in it: but if this is so, the validity of our chief conclusion is not affected, opportunity for such participation being equal in the two types of experiments which are compared.

In two conspicuous instances (urea and phenol red), evidence is at hand that the injected substance passes not only into but through the cells so that neighboring tubules are reached. The high degree of diffusibility

of urea is well known: experiments which we have made on diffusion of various dyes into gelatin show that phenol red is highly diffusible.[2]

Our results indicate that greater emphasis may properly be laid upon certain discrepancies which are encountered in the work of others when cell deposition is compared with urinary elimination. Carmine appears in the urine before detectable granules of red can be found in the tubule cells: iron and trypan blue are to be found in tubule cells long after urinary elimination has ceased. They indicate also that it may be futile to attempt, as Gurwitsch and Stieglitz have done, to judge of direction of passage of substances through renal cells by the positions in which colored granules are to be found at various times after injection. Stieglitz found prussian blue granules more abundant near the basement membrane in kidneys taken two minutes after injection than in those taken ten or more minutes after: yet in one of our experiments a kidney taken immediately at the end of an intratubular injection lasting only 20 seconds showed granules of prussian blue close to the basement membrane.

It is noteworthy that the colloidal dye, trypan blue, passes through the glomerular membranes; for its blue component does not pass through an ordinary collodion membrane.

## SUMMARY

1. The following substances have been introduced directly into the lumen of the renal tubules in living frogs by means of a capillary pipette thrust through the capsule of Bowman into the intracapsular space: indigocarmine, sodium carminate, methylene blue, toluidin blue, trypan blue, ferric ammonium citrate, urea. The amount so injected was too minute to be capable of producing any demonstrable effect if injected into the blood stream. In every instance the injected substance was identified within the cells of the injected tubule, distributed in manner and amount comparable to that found when it is introduced in large amounts into the circulating blood.

2. Each of the substances named has been found to be present in the glomerular fluid after it has been introduced into the circulating blood.

3. The conclusion is reached that results derived from the microscopic study of fixed sections of the kidney taken after intravenous injections of microscopically identifiable substances can not form valid evidence of

[2] Shimidzu (1922), in his study of the permeability of the rodent placenta estimated the rate of diffusion of various dyes into solid gelatin (20 per cent). Selecting from his table those which we have used in these experiments, the order of decreasing diffusibility is: methylene blue, toluidin blue, indigocarmine, trypan blue. We have made similar experiments with confirmatory results; and in addition have found the diffusibility of phenol red to be nearly twice as great as that of methylene blue.

secretory activity of renal tubules, except in the case of substances which can be proved to be present in the urine without having passed through the glomerular membranes. At present we do not know of such substances.

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# THE EFFECTS OF INJECTING ANTERIOR HYPOPHYSIAL FLUID ON THE COURSE OF GESTATION IN THE RAT[1]

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The daily intraperitoneal injection of anterior hypophysial fluid into adult female rats has been shown to produce, in addition to acceleration and prolongation of growth, striking changes in the ovary. Immature follicles develop lutein tissue without rupture and expulsion of the ovum. The vagina and uterus cease to undergo the rhythmical changes which have been found (8) to accompany ovulation, or at least they undergo these changes only at very lengthy and irregular periods; indeed ovulation itself rarely occurs.

Evans and Long, in their monograph (6), have worked out in detail the normal sequence of events in the gestation of the rat. Females ovulate every $4\frac{1}{2}$ days and oestrus may be detected simply and precisely by means of a microscopic examination of a smear taken from the vagina.

Females to be bred are chosen at oestrus as determined in the manner mentioned above and placed with a male in the evening. Whether they have accepted the male is determined the following morning by the demonstration of residual sperm in the vaginal content. The day on which sperm are found is called day 1. On day 6, in the normal gestation, implantation occurs. A "placental sign," which consists of the appearance of red blood cells in the vagina, occurs on days 13 or 14, and only in rare cases as late as day 15. These red blood cells are presumably the result of a leakage from the placenta when the young have reached a certain definite stage of development. The normal pregnant animal is isolated on day 21, and the young are almost always born on day 23, though occasionally on day 22.

The animals used in these studies were females of proven fertility, i.e., they had given birth to at least one normal litter. They were kept under the best of hygienic conditions and were maintained on an ample diet during the course of the experiment.

The fluid used for these injections was made from the fresh bovine

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hypophyses. The dissected anterior lobe was partially sterilized by subjecting it to 40 per cent alcohol for 10 minutes. It was then triturated with sterile sand in a sterile mortar. The paste obtained was extracted overnight on ice with an equal volume of sodium hydroxide, the final concentration of the preparation being 0.054 N. The following morning the preparation was neutralized to phenol red with 0.2 N acetic acid. The neutralized extract was then centrifuged at high speed for ten minutes, the supernatant fluid being used. At the time these experiments were begun, this fluid was more potent and constant in effect than any which had previously been prepared as shown by acceleration of growth and inhibition of oestrus in rats (4).

EXPERIMENTS. *1. Observations on the living animal.* Normal females were selected which had never been treated with hypophysial fluid and which had been mated positively, as was shown by the demonstration of

TABLE 1

| ANIMAL | DAY OF APPEARANCE OF RED BLOOD CELLS | FIRST DAY OF DECLINE IN WEIGHT |
|---|---|---|
| GH9105 | 15th day | 25th day |
| B7143 | 15th day | 23rd day |
| BH9540 | 16th day | 25th day |
| BH17 | 18th day | Gaining when autopsied on 25th day |
| W47 | 18th day | 26th day |
| BH6551 | 19th day | 29th day |
| W8716 | 19th day | Gaining when autopsied on 25th day |
| B69 | 20th day | 27th day |

residual sperm in the vaginal canal on the morning after having been placed with a male. From the first day they received intraperitoneal injections of 1 cc. of anterior hypophysial fluid daily.

There were no apparent deviations from the behavior of the normal pregnant animal during the early part of the gestation except that these injected animals immediately began to gain in weight. Quite uniformly they averaged a gain of 40 grams during the first ten days of gestation, whereas the normal animals show only a slight gain, if any, during this period.

The first disturbing deviation from the normal was that the "placental sign," i.e., the presence of red blood cells in the vagina, failed to appear at the customary time, viz., day 13 or 14, except in a small minority of cases. However, careful daily examination of the animals revealed the appearance of this sign later, varying from day 15 to day 20 or still later. Commonly it occurred on day 18 or 19.

The injected animals continued to gain in weight and were isolated on day 21. The young of normal animals, as previously mentioned, are born

on day 23 but with these injected animals no litters were born on day 23 and many of the pregnant animals continued to gain in weight until day 27 or 28, after which they usually came to a plateau for 24 to 36 hours and then lost in weight. The day on which the animals first lost weight was usually well correlated with the date of appearance of the placental sign as shown in table 1. In each case on days 26 to 30 stillborn fetuses were expelled.

From table 1 it will be seen that the "placental sign" seems to signify that a definite degree of development of the fetus has occurred in the injected animals, just as in the normal animals. The stillborn fetuses were invariably partly eaten by the mother and were not weighed but they appeared to be of full term development and, on the average, of greater size than the normal. As mentioned subsequently, this observation was confirmed by the actual weights of term fetuses removed at autopsy after similar treatment. In no instance in which an animal was injected throughout the gestation period was there a live fetus born.

In five cases resorptions were suspected of fetuses of somewhat more than half-term development. In four of these cases autopsies confirmed these suspicions. However, in view of the facts that resorption of fetuses by normal animals is not uncommon and that resorptions in these experiments were so few (4), resorption is not believed as yet to be a usual or specific effect of anterior hypophysial fluid.

These observations on the living animal may be summarized as follows:

1. The course of gestation in animals injected daily throughout the term with anterior hypophysial fluid is lengthened from two to six days.

2. For some reason, as yet unexplained, the young invariably die, after reaching term development, and are stillborn.

3. The stillborn young would appear to be of a larger size, on the average, than the normal.

4. Resorption of fetuses which have not reached term development occurs in a minority of the cases.

*2. The cause of the lengthening of the gestation.* In seeking the cause of the lengthening of the gestation period of animals injected with hypophysial fluid, it was thought best, in view of the delayed "placental sign," first to study the uteri of injected animals in the early days succeeding the normal implantation day (no. 6). Accordingly, four animals were sacrificed on day 9. The uteri in each case showed no gross evidence of implantation. Serial sections of parts of the uteri of three of these animals were made. In one no eggs were found. In the others blastocysts resembling the six-day blastocyst described by Huber were found free in the lumen of the uterus without the slightest evidence of a placental reaction on the part of the uterus.

Of five animals sacrificed on day 10, in three no implantation sites were

to be seen; in two, sites of from eight to nine days' normal development were seen.

Of four animals killed on day 11, three showed very minute sites of perhaps the normal development late on day 6, and one showed no certain evidence of implantation (see table 2).

TABLE 2

*Autopsies to determine day on which implantation occurred*

Animals injected from day 1 to date of autopsy. (Normal implantation day 6)

| ANIMAL | KILLED ON DAY | IMPLANTATION SITES | MICROSCOPIC |
|---|---|---|---|
| W01 | 9th | None | No blastocyst located in serial sections of part of uterus |
| W8572 | 9th | None | Blastocysts of approximately six days' development lie unimplanted in the uterus |
| BH8196 | 9th | None | Same |
| GH9103 | 9th | None | Not sectioned |
| W9687 | 10th | Nine sites resembling normal eight-day | |
| B6564 | 10th | None | Not sectioned |
| W1212 | 10th | None | Not sectioned |
| GH7594 | 10th | None | Not sectioned |
| W9613 | 10th | Eight sites 2x3 mm. | Not sectioned |
| W70 | 11th | No sites of implantation observed | Not sectioned |
| B7845 | 11th | Eight very minute enlargements | Normal 7-day blastocysts |
| GH7923 | 11th | Twelve minute enlargements seen | Not sectioned |
| BH69 | 11th | Six very slight enlargements | Not sectioned |
| W00 | 12th | Eleven sites varying from 3 x 4 to 4 x 5 mm. | Anterior portion of neural tube nearly closed (normal day 9) |
| W7171 | 12th | Eleven sites, 4 x 5 mm. | Anterior portion of neural tube closed (day 9) |
| W9539 | 12th | Five sites; two are 4 x 5 mm., three very slight enlargements | Embryo of day 9 |
| G4097 | 12th | Two sites; 2.5 x 3 mm. | Egg cylinder stage (day 7–8) |

Four animals were sacrificed on day 12. In each case, implantation sites grossly resembling normal sites of days 7 or 8 ($2.5 \times 3$ mm. to $4 \times 5$ mm.) were found. Serial sections of these sites revealed embryos which varied from the egg cylinder stage to one in which the anterior part of the neural tube had closed.

From these observations on the cause of delay in gestation it may be stated that:

1. The eggs do not usually become implanted in the uteri of injected animals until days 10 or 11.

2. This delayed implantation is a sufficient explanation of the delay in the gestation.

*3. The termination of gestation with the death of the fetus.* Although the preceding section has shown that a lengthening of the gestation period occurs, due to a delay in implantation, there is as yet no evidence which would indicate that the fetus is injured until the very last part of gestation.[2]

For example, the uterus of an animal (W 7739) killed on day 20, which had not yet shown the "placental sign," contained apparently normal living young of 7 mm. or 14 days' normal development (fig. 1). A second animal (B H 17) killed on day 25, had, *in utero*, living young of approximately 18 days' normal development; these young appeared to be perfectly normal. In a third animal (B 69) killed on day 27, were found living and vigorous young of 21 days' normal development. The average

Fig. 1. Photograph of fetus (magnified 5 diameters) removed from the uterus of an injected animal on day 20. Note the rudimentary states of the limb buds and head. The fetus was of fourteenth-day normal development. (Both sides are shown.)

weight of the young in this case was 6.1 grams, which is considerably above the average weight for normal term fetuses which is 5.8 grams. This difference in weight is considerably greater than is at first apparent in that the former were only of 21 days' normal development, whereas the figure of 5.8 grams is the weight of term fetuses of 23 days' normal development. Sections from the placenta of these young showed no microscopical evidence of a lesion. Finally, an animal (W 1373) which had lost weight over-night was sacrificed. The uterus of this animal contained eight fetuses, of which five were dead; three of these were being resorbed and two were of term development. Three other fetuses were still living

[2] It is true that a small number of early resorptions have occurred, but in view of the infrequence of these resorptions and the fact that resorptions are not infrequent in normal animals, it is not felt that such a course could be considered a specific effect of anterior hypophysial fluid.

though there was blood in the amniotic cavities of these young as well as in those of the two term fetuses which were already dead.

The three living fetuses which were definitely sick were removed and, after being warmed, they were placed with a litter born on the same morning of a normal female. The following morning they had completely recovered and appeared as vigorous as the normal young of the litter. After 35 days these young are quite as healthy as any normal animals.

In striking contrast to the normal picture to be found in an injected animal sacrificed when the young are a few hours under term development, is the picture seen when the injected animal is not killed until she has remained stationary or lost weight for 48 hours. The young of such an animal are always dead, though of term development, and greater than normal weight. The average weights of term fetuses from the litters of eight animals in which the fetuses were removed at autopsy was 6.6 grams, as opposed to 5.8 grams average for the normal stock colony. The placenta is commonly hemorrhagic and displaced somewhat from its uterine attachment and a large amount of blood lies free in the amniotic cavities of the young.

In a number of instances animals were fortunately sacrificed at a time when some of the young were living while others, often in adjacent sites, were dead. Such, for example, was true of an animal (G H 9105) which was injected from day 1 until day 23 and autopsied. The left uterine horn contained five living fetuses and one dead one. The dead fetus weighed 6.25 grams and was obviously of term development. The right uterine horn contained five living fetuses. The amniotic cavities of all the live fetuses, as well as the dead one, were heavily tinged with blood. In four similar instances dead young were found in amniotic cavities which contained blood, while the placentae were quite definitely dislodged from their uterine attachments. Of the living, some were nearly dead while others appeared to be normal. In some the amniotic fluid was hardly tinted with blood, while in others there was quite extensive hemorrhage. These were clearly examples of an intermediate stage between the normal conditions existing a few hours before term and one in which the fetuses were found dead and the amniotic cavities full of clotted blood.

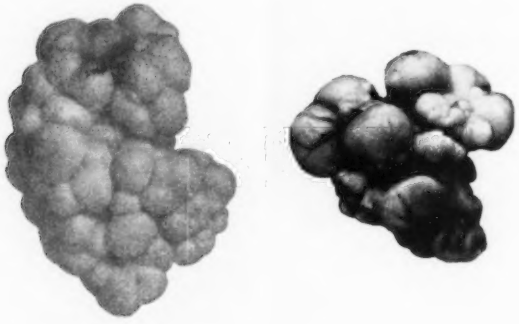
These findings may be summarized as follows:

1. The fetus of the injected mother reaches term development but is invariably stillborn.

2. The average weight of fetuses taken at the time of weight-decline from the uteri of injected animals is distinctly greater than normal (6.6 grams as opposed to 5.8 grams).

3. The fetuses of injected animals are normal at term and probably die because of a failure of the birth mechanism, the precise cause of which is as yet unknown.

*4. The rôle of the ovary.* Invariably associated with the conditions described above are remarkable changes in the ovary. It is always large, both relatively and absolutely. In ten of the injected animals the average weight of the ovary was 126.1 mgm. whereas the normal ovary of a pregnant animal at term is 75 mgm. Grossly the ovary of the injected animal appears to be made up almost entirely of corpora lutea, many of which are very large (fig. 2). As many as twenty large corpora lutea have been counted on one surface of such an ovary. The "corpora of pregnancy" cannot with certainty be distinguished from those which arise from the hypophysial fluid stimulus.



Experimental Control

Fig. 2. Photographs (magnified 5 diameters) of an ovary removed from a pregnant rat at term. This animal received injections throughout the gestation. Note the exceedingly large number of corpora of varying sizes. The ovary of a normal pregnant animal at term is shown for comparison.

Microscopically there are seen a great number of corpora of varying sizes in the centre of many of which are ova. Some corpora which are very large and show no ova are perhaps corpora of pregnancy.

In the animals treated throughout with fluid, no follicles have been observed. The only difference in appearance between the ovary described above and the characteristic "mulberry ovary" produced by Evans and Long by injecting virgin females with hypophysial fluid is that the former is considerably larger (8).

It was thought possible that the peculiar ovary produced by this treatment might in some way be more directly concerned with the aberrations shown by the pregnant animals than the fluid itself; that perhaps the hypophysial fluid acted "through the ovary," so to speak. This was put to experimental test as follows.

In view of the fact that implantation normally occurs on day 6, five positively mated animals were not injected until day 6, on and after which they were injected daily throughout the term, with the hope that the delay in the gestation period might not occur. One showed red blood cells on day 14, three on day 15, and one on day 16; in other words in all but one case the "placental sign" appeared within the normal limits. These young came to term uniformly earlier than animals injected from day 1, as indicated by loss of weight on days 23 to 24. All animals were sacrificed on day 25. In four cases all the young were dead and appeared to have been so for 24 to 48 hours. In one case there were six dead fetuses and one was still alive, though the usual hemorrhage in the amniotic cavity was extensive. So it was found that though implantation was not delayed, the normal birth of the fetus was interfered with.

TABLE 3

| ANIMAL | PLACENTAL SIGN (NORMAL—DAY 13–14) | DAY OF LITTER (NORMAL—DAY 23 | NUMBER IN LITTER |
|---|---|---|---|
| W6558 | Day 18 | Day 27 | 10 |
| BH6552 | Day 18 | Day 26 | 7 |
| BH7208 | Day 16 | Day 25 | 9 |
| G9111 | Day 18 | Day 25 | 10 |
| W7253 | Day 19 | Day 26 | 10 |

Five animals were injected from day 1 to day 12 only, with the hope that implantation might be delayed and that the animal still might "recover" from the effects of the injection by the time the young had come to term. The "placental sign" was late in all cases but only in one case was a litter of live young born. This litter of 13 small (av. 5.4 grams) but healthy animals was born on day 27, four days after the normal term. One would not expect them to be of large size because injections were stopped at about the time they became implanted (day 12). One of the other animals had a stillborn litter of 12 on day 28. The other three animals were sacrificed on day 27. Some of the young were dead in each case, while others were living but with the characteristic lesion, hemorrhage into the amniotic cavity. The persistence of this failure to give birth to the term fetuses, even though the animals had not been injected for 12 to 15 days, would pointedly suggest a specific effect of the treatment. The ovaries in these cases retained their "mulberry" characteristic, containing many large corpora lutea. In one case, a few small follicles had appeared. Unfortunately the ovaries of the animal which had a living litter could not be studied as the litter was permitted to lactate.

Five animals were injected from day 1 to day 6 inclusive, after which injections were stopped for the remainder of the period. Every animal

had a living litter though the gestation period was lengthened in each case from two to four days, as shown in table 3.

It is of interest to observe that ovulation in the case of virgin animals injected for a short period of time with anterior hypophysial fluid does not return until after injections have been discontinued for from 10 to 15 days; another instance of the persistence of the effects from this treatment. It is also to be noted in the group of animals injected for 12 days, leaving 13 to 15 days to recover before parturition, that only one had a live litter, whereas in the last group injected for only six days, which had 19 to 21 days to recover, every one, even though the gestation was lengthened, had a large litter of healthy young.

It is to be noted that of the five animals injected for the first 12 days one gave birth to a live and healthy litter on day 27 and that the others failed to give birth to living litters. It will also be observed that there is a fairly wide variation in the number of days of delay in the gestation of animals injected throughout its course. This variation in degree of effect might be assumed to be in accord with the results from injection of virgin females in which there is a wide variation in the amount of acceleration of growth produced, as well as in the degree to which ovulation is affected for individual animals. In a small minority of these animals 0.25 cc. of the fluid daily produces a very marked acceleration of growth and complete inhibition of ovulation; while in others 1.0 cc. daily, while it always appreciably accelerates growth and affects ovarian rhythm, may not produce in some animals as marked effects as 0.25 cc. causes in others. One is inclined to ascribe these variations to individual differences in sensitivity.

Four animals were injected from day 17 to term; none of them littered on day 23. They were autopsied on day 24 and in each animal some of the fetuses were dead while others were living. There was blood in the amniotic cavities of all, and in one case (W 76) the placentae of several of the fetuses were definitely dislodged. The ovaries in each case, in addition to the corpora of pregnancy, showed many smaller and vascular corpora, obviously the result of hypophysial fluid.

This last group of experiments shows that:

1. The delay in implantation, or the death of the fetus at term, may be produced independently of each other.

2. The condition of the ovary is in some way associated with the failure of the birth mechanism.

Discussion. J. F. Daniel first reported a lengthening of the gestation period in white mice which resulted when an animal was suckling one litter while carrying another. The delay varied from one to eight days and appeared to vary distinctly with the number of young suckled and the number carried. The cause of this delay was not investigated but it was

proposed that lactating one group of young might deprive the carried group of sufficient nutrition and that for this reason the young carried might develop more slowly. H. D. King confirmed these observations on the rat (11).

The cause of this delay in the gestation period was revealed by W. B. Kirkham (12) to be principally, if not entirely, due to delayed implantation. It was also shown that the blastocysts lay free in the lumen of the uterus from day 6 to day 14, when they were implanted. However, Kirkham did not offer an explanation for this delay. No other report of delayed implantation has been observed.

It is not believed that malnutrition could have been responsible for the delay in implantation in the experimental animals here reported upon. The animals received an abundance of properly balanced food and gained remarkably in weight during the first 10 days of this period. With reference to the work of Kirkham, it may be said that it is well known that ovulation occurs within 24 hours after parturition. It is also known that provided the mother is permitted to suckle her young, the corpora from this ovulation persist as the so-called corpora of lactation. If one were to assume that these corpora are in some way concerned directly, or indirectly, in making lactation possible, it might be said that the corpora of animals lactating one litter while carrying another are performing a double function; at least, corpora which are ordinarily concerned, or better associated, with lactation only, are here concerned with implantation as well. As has been pointed out, the ovaries of pregnant animals injected with anterior hypophysial fluid also contain lutein tissue which is not present normally during the course of gestation. It is felt that the presence of this lutein tissue not ordinarily concerned with gestation, both in the experiments of Kirkham and in these is more than coincident. It must be acknowledged that as yet this point is not established.

Three possibilities present themselves. The delay in implantation may be due: 1, to toxicity or an injury to the egg; 2, to a lack of sensitivity on the part of the uterine mucous membrane at the time when the egg first appears in the uterus; or 3, the egg may not reach the lumen of the uterus on days 4 to 5, as is normal.

The first explanation would appear improbable in that the eggs had burst from the follicles before injections were begun and it would seem to be somewhat far fetched to assume that the fluid in which they are bathed in the tube might exert such an influence and yet permit them to implant later. The second possibility while not altogether untenable is open to a perplexing objection. It has been shown that if virgin females are injected daily with anterior hypophysial fluid, injury to the uterine mucous membrane results in a decidual cell response (placentoma). This response results even though the injury to the uterus be made as early as the day of

the sixth injection. The placentoma resulting from such an injury to the uterus is histologically identical with the response to normal implantation. If this placentoma reaction might be regarded as conclusive evidence that the uterus is sensitized to the implantation reaction, on day 6, one might rule out the second explanation. However, mechanical injury to the uterine mucous membrane might be a considerably stronger stimulus than the presence of a fertilized ovum.

The third explanation, that the egg might be delayed in reaching the lumen of the uterus, appeared a possible one. It was thought that the same phenomena which make a normal birth in these injected animals impossible might possibly be concerned with a delay in propulsion and distribution of the eggs along the uterine horns. Reference is here made to the possibility of a change in the contraction phenomena of the tubes and uterus.

Six animals were injected from day 1 to day 6 inclusive, and autopsied. Serial sections of the tubes of two of these animals do not reveal any eggs. Eggs were located free in the lumen of the uterus in one of these cases. An attempt will be made to locate them in each case. The evidence from sections of the tubes of the two animals as yet cut would tend to show that the condition here is similar to that found by Kirkham; namely, that the egg reaches the lumen of the uterus before day 6, as is normal, but lies free and unimplanted for from two to six days.

It has been observed by clinicians that conditions in human beings which are doubtless due to hyperactivity of the anterior hypophysis (gigantism and acromegaly) are sometimes accompanied in the male by excessive libido sexualis and potency. Curiously enough the opposite is the case in the female: one of the first signs of hyperactivity being amenorrhea. It is, of course, well known that hyposecretion also results early in amenorrhea in women, and loss of sex vigor in the male. The occurrence of amenorrhea in both hyper- and hypoactivity of the anterior pituitary in the female has been difficult to explain.

In the case of hypoactivity the amenorrhea may be explained as the impotence of the male by the resultant atrophy of the gonad. Amenorrhea and impotence have been two of the most constant effects obtained by injury and removal of the gland. It is not believed that the amenorrhea resulting from hypersecretion is due to ovarian atrophy. It is more probable that it results from the production and the persistence of lutein tissue in the walls of immature follicles. The continuous presence of lutein tissue might well inhibit ovulation and menstruation.[3] Loeb,

[3] Walker (14) from studying the effects of injections of anterior hypophysial fluid in the chicken came to the conclusion that it is the ovum which is primarily destroyed through a specific toxic effect in birds, and questions the original view of Evans and

Frank, Evans and others have shown that the lutein tissue which persists in pregnancy and lactation is responsible for the inhibition of ovulation and the rhythmetic changes in the genital tracts of animals during these periods, which are in a sense comparable to menstruation. The occurrence of this lutein tissue in animals injected with anterior hypophysial fluid is coincident with the disturbance and cessation of ovulation and the oestrous cycles. After injections have been stopped, ovulation and oestrus do not occur until the lutein tissue formed from injections disappears. This is a matter of 12 to 20 days after injections are stopped. It would seem reasonable that the amenorrheas of hypo- and hyperactivity are due to totally different conditions in the ovary: the former to ovarian atrophy and the latter to the production and persistence of a large amount of abnormal lutein tissue which results in a great increase in the size of the ovary. This ovary of hypersecretion is not incompatible with the implantation and nutrition of fetuses and the probable reason why the young of the animals described in these experiments were not born alive is that the persisting lutein tissue, which disappears during the latter part of the normal gestation, inhibited the maturing of Graafian follicles. The ovary might in a sense even be called hyperactive, though of course the function of gestation is clearly and disastrously affected.

In connection with the possible association of the peculiar condition in the ovary at the end of the gestation with the inability of these animals to give normal birth to their young, Dixon has recently shown that in normal pregnant animals the injection of extracts of ovaries from other animals at about oestrus will hasten parturition. However, extracts of ovaries containing only corpora lutea and no mature follicles do not produce such an effect. As has been pointed out, the ovaries of the pregnant animals injected with anterior hypophysial fluid contain only corpora and no mature follicles (fig. 2). Normal pregnant rats as well as many other species of animals, ovulate within a few hours after parturition. The injection of anterior hypophysial fluid does not permit the ripening of follicles. It is very possibly for this reason that normal parturition does not occur.

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Long (7), (8) that the anterior hypophysial fluid exerts a powerful and specific stimulus to lutein tissue formation.

From the experiments presented in this paper it is certain that the fluid is not toxic, nor directly injurious to the developing blastocyst and fetus. Further, animals whose ovulation has been stopped for months by injections of hypophysial fluid will resume ovulation after injections have been discontinued. If Walker is correct in his view that the fluid is specifically toxic to the ovum it could only be so to the maturing cell: that is to say, long-continued injections do not destroy the primordial ova.

It might be objected that the effects on gestation elicited with this treatment are not specific, either as a direct hypophysial effect or as an indirect effect through the ovary.

It has been proved that 0.5 N NaOH will in 12 hours destroy the active principle or principles of hypophysial fluid. The control fluid was made with anterior hypophysial substance extracted with 0.5 N NaOH but otherwise prepared in exactly the same way as the active solution. It is thought that this preparation is an ideal control fluid as it contains the same proteins as the active solution, and differs from it only in the destruction of the hormone and a high NaAC content.

Three animals were injected with this control solution from day 1 and were sacrificed on day 7. In each case implantation sites, identical with those in the 7-day normal animal, were found.

Four animals were not injected until day 16, after which injections were continued until day 23 when each animal had a normal litter. So it would appear that the control injections caused no disturbance whatsoever in gestation.

## SUMMARY

The gestation of pregnant rats which are injected with anterior hypophysial fluid from the day after they accept the male until term exhibits the following deviations from the normal:

1. The gestation period is lengthened from two to six days; in other words, term development which normally is reached on day 23 is delayed until day 25 to 29.

2. This lengthening of the gestation period has been totally accounted for in a corresponding delay in implantation: that is, implantation, which normally occurs on day 6 has been shown to occur on days 9 to 12.

3. Term fetuses invariably die *in utero*, and after lying in the uterus for from 24 to 48 hours, are expelled stillborn.

4. The death of the term fetuses *in utero* is due to a failure of the birth mechanism of the mother. The placenta is slowly dislodged and the young quite possibly die of asphyxia.

5. That the injury is not primary in the fetuses has been shown by removing them from the uterus at term and rearing them with a foster-mother.

6. The persistence of abnormal lutein tissue, which is a specific effect of anterior hypophysial fluid, is always associated with the failure of the birth mechanism. It is thought that the persistence of this lutein tissue is directly responsible for the inability of the mother to give normal birth to her fetuses.

7. The average weight of term fetuses of injected animals is greater than that of normal term fetuses, 6.6 grams and 5.8 grams respectively.

The writer wishes to express his great obligation to Prof. Herbert M. Evans for his suggestions and guidance in the conduct of these studies, and to Prof. Harvey Cushing for the opportunity of continuing the work in his laboratory in Boston.

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# THE EFFECTS OF INJECTING ANTERIOR HYPOPHYSIAL FLUID ON THE PRODUCTION OF PLACENTOMATA IN RATS[1]

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Evans and Long (3) in their monograph on the oestrous cycle in the rat, described a group of experiments on the production of placentomata. They found that during the normal 4½-day cycle there was no time at which injury to the uterine mucous membrane provoked a decidual cell response. These results appeared to be contradictory to those of Corner and Warren (1), Robert Frank (6) and Leo Loeb (7). However, Corner and Warren, and Frank, all of whom worked on the rat, took the ovulation which was known to occur within twenty-four hours after parturition in calculating the age of the corpora, which corpora it would appear are the sensitizing agents of the uterine mucous membrane. It has since been shown that these corpora occurring after parturition endure for a considerable period as the so-called corpora of lactation. Leo Loeb's experiments, of course, were performed on the guinea pig in which the normal cycle is 18 days. Evans and Long based their failure to produce the reaction during the normal cycle in the rat on the fact that the normal cycle is so short that by the time the corpora resulting from one ovulation have attained a functional age (3 to 4 days) even though the injury be made, the following ovulation which would occur within 24 to 36 hours would effectively destroy any beginning decidual cell response. However, they were successful in producing placentomata during the diestrum of 10 to 11 days which occurs in the condition of pseudopregnancy.[2]

The corpora which Evans and Long produced in virgin female rats by

[1] Aided by grants from the Committee for Research in Problems of Sex of the National Research Council, administered by Dr. Herbert M. Evans.

[2] The condition which Evans and Long described as pseudopregnancy may be induced by mating a female at oestrus with a vasectomized male, or more simply by inserting a foreign body such as a fine glass rod into the cervix momentarily at oestrus. A female thus treated behaves for 10 or 11 days as a pregnant animal. The mammary glands show hypertrophy and the corpora persist. The whole genital tract is strikingly similar to that of the pregnant animal. On the 12th or 13th day ovulation occurs after which normal cycles are again resumed.

daily injection of anterior hypophysial fluid appeared, at least morphologically, to be functional. However, they had obtained no experimental evidence on the point.

It was thought possible to determine whether these corpora produced by

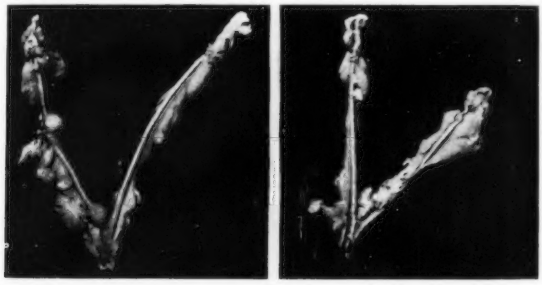

Fig. 1. Photographs (½ diameter) of complete genital tract, showing placentomata produced in an injected animal. Photograph (on right) of control with no swelling.

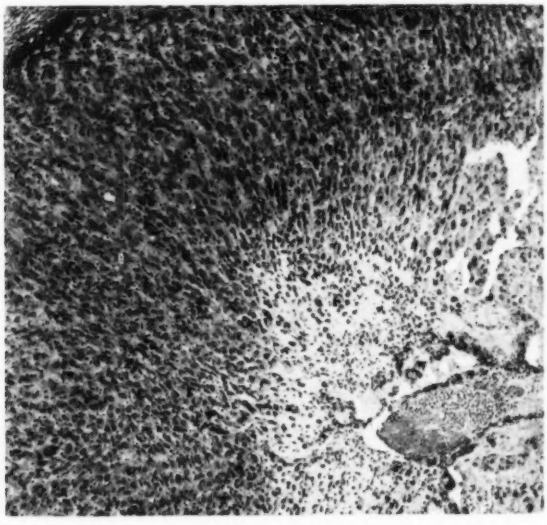

Fig. 2. Microscopical section through one of the placentomas shown in figure 1

anterior hypophysial fluid were functional as regards their ability to sensitize the uterine mucous membrane as determined by the placentoma reaction of Leo Loeb (7). It was also thought possible to determine

whether the uterine mucous membrane of injected animals was capable of exhibiting the implantation reaction as early as day 5 or 6.

EXPERIMENTS. *Group I.* Six adult females, which were having normal cycles, were chosen at random with reference to the stage of the cycle. They were injected daily with anterior hypophysial fluid throughout the experiment. On the day of the fifth injection, injuries to the uterine mucous membrane were made. These injuries were produced after the method described by Evans and Long: namely, by inserting a loop of black silk thread, loosely tied, through the lumen of the uterus. On the sixth day after the operation these animals were sacrificed. The ovaries in each contained numerous relatively large corpora. In every animal large tumefactions (some measuring over 1 cm. in diameter) were found at the sites of injury (fig. 1). Microscopical sections from the tumors of each of these animals showed a vigorous and typical decidual cell response (placentoma) (fig. 2).

Two sets of control experiments were made.

*Control group I* consisted of six animals in which, without injections and without regard for the stage of the cycle, injuries were made to the uterine mucous membrane similar to those described above. These animals likewise were killed on the sixth day following the operation. In five of these animals only a few small corpora were to be made out grossly and in one (B 1336) eight uniform-sized corpora of about 0.6 mm. in diameter were seen. In no case, either grossly or microscopically, was there any evidence of a decidual cell reaction.

*Control group II.* Six animals were injected daily throughout the experiment. On the fifth day of injection, injuries were made to the uterine mucous membrane as in the preceding two groups, but both ovaries were removed. On the sixth day after operation the animals were sacrificed. In no case was there either gross or microscopic evidence of a placentoma.

The first group of control experiments was simply a repetition of the work of Evans and Long, and confirms it. In the second group, it was intended: primarily to determine whether anterior hypophysial fluid is a stimulus to decidual cell reaction whether or not the ovary be present; secondarily, whether hormones due presumably to the corpora might nevertheless have remained in the circulation and have exerted a sensitizing influence even after ovariectomy.

It was realized that should these tumors be produced in the absence of the gonads, one should still admit the possibility of a temporary survival of ovarian, presumably luteal, hormones in the circulation. The outcome, however, obviated all necessity of such an admission. Since in the experiments described, one of the chief effects of anterior hypophysial fluid was the over-production of lutein tissue in the ovaries, one might look on the placentomata as conditioned by lutein hormones thus continuously present. It may be recalled that the lutein tissue in these cases is not merely present in the corpora which form after ovulation, but that this tissue also is seen forming in the walls of unruptured follicles imprisoning the ova.

Among the corpora which result from the hypophysial stimulus there must of course be considered that group which has arisen from the last

true ovulation preceding the first injection. From these experiments it cannot be determined whether the sensitizing influence exerted is not due entirely to this particular group of corpora. However, from the fact that in another case in which corpora were produced in an animal too young to have ovulated and in which the decidual cell reaction was obtained, it is believed that the peculiar corpora produced by hypophysial stimulus would have to be considered functional in this respect.

## SUMMARY

1. Under the conditions described, the daily injection of anterior hypophysial fluid always brings about a typical decidual cell response to injury of the uterine mucous membrane in animals in which the ovaries are present.

2. This response does not occur when the ovaries are absent.

3. Provided that this decidual cell reaction is indicative of the ability of the uterus to implant the egg, it would not appear that the cause of the delay in implantation in injected pregnant animals is due to a lack of sensitivity of the uterine mucous membrane on day 5 or 6.

As in the previous paper, the writer wishes to express his great obligation to Prof. Herbert M. Evans and to Prof. Harvey Cushing for their suggestions and encouragement in this work.

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# SUPERIMPOSED RESPIRATIONS OR CHEYNE-STOKES BREATHING OF AMPHIBIOUS AND NON-AMPHIBIOUS MAMMALS

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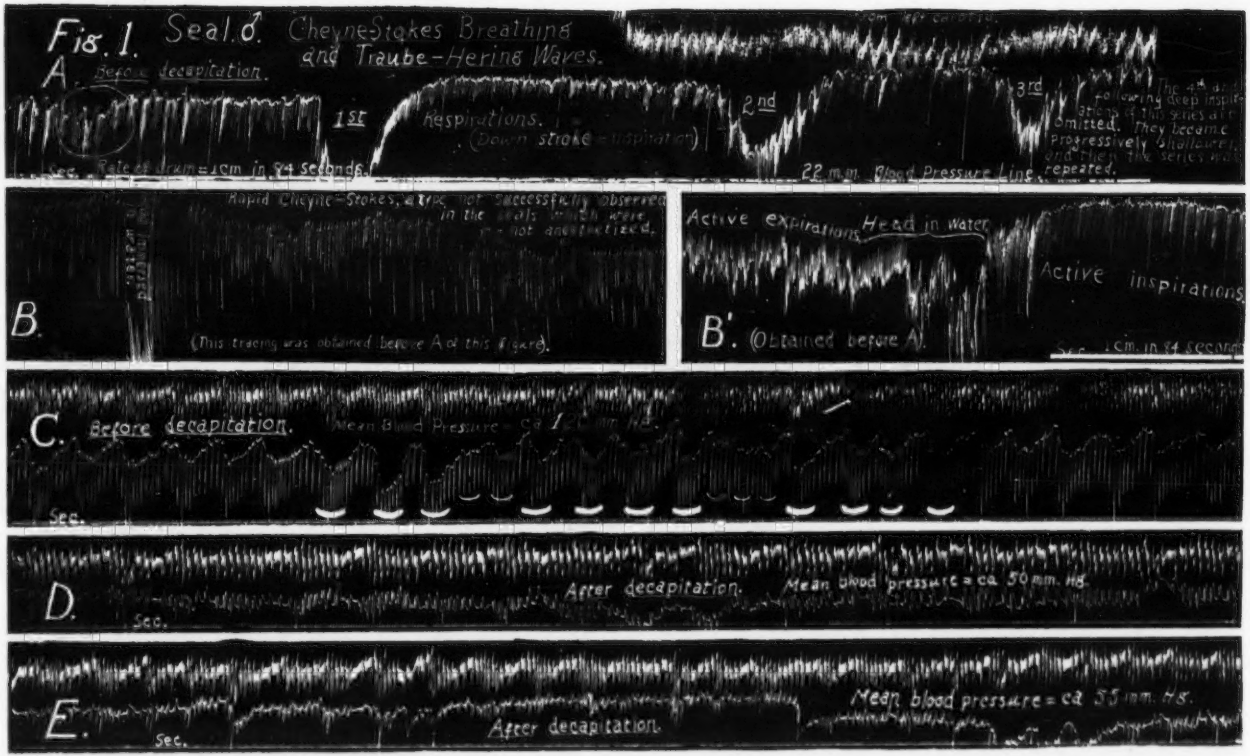
Anesthetized dogs and cats were trained to breathe in the Cheyne-Stokes manner (Swindle, 1925). The negative pressure in the chest was slowly and systematically altered without interfering greatly with the relatively rapid, spontaneous breathing. After an hour or more of this training, the slow, artificial respirations were replaced by spontaneous ones of similar duration. The rapid and slow respirations were then superimposed, and the breathing was accordingly of the periodic or Cheyne-Stokes type.

Further evidence that the respiratory mechanism can be trained to react in complex ways was sought from certain mammals whose natural behavior is such that they are subjected during much of their lives to a presumably very efficient training which should cause them to breathe habitually in the Cheyne-Stokes fashion. These are such amphibious mammals as the hippopotamus, polar bear, seal, mink and muskrat. These animals often submerge the nostrils at fairly regular intervals. If an anesthetized dog or cat can be trained in an hour or two to manifest superimposed respirations, the aquatic mammals should be expected to be habitual Cheyne-Stokes breathers, as they are in fact (figs. 1, 2 and 3).

An observation which proved to be of considerable importance in the present study is that the aquatic mammals often execute respiratory-like movements while under the water. These are either simple abdominal or thoracico-abdominal reactions, and both varieties are usually more rapid than the apparently more normal thoracico-abdominal respirations which take place while the animals are out of the water. Even the strongest of these under-water movements very seldom cause bubbles to rise rhythmically from the firmly-closed nostrils. However, when such an animal as the seal is tracheotomized when the under-water movements occur, each of these creates a current of air in the tracheal cannula. Now movements like these are manifested by the decapitated kitten (fig. 4) and are effective respirations; they may suffice to keep the animal alive for a

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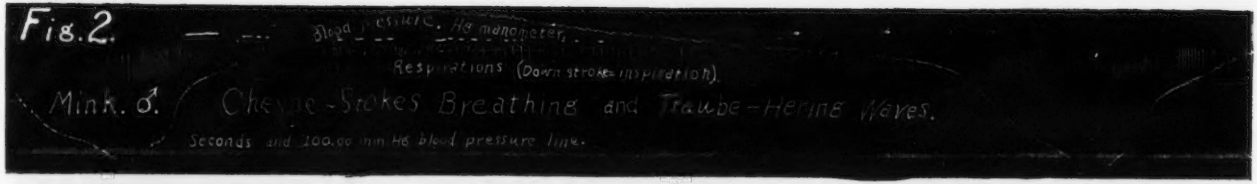
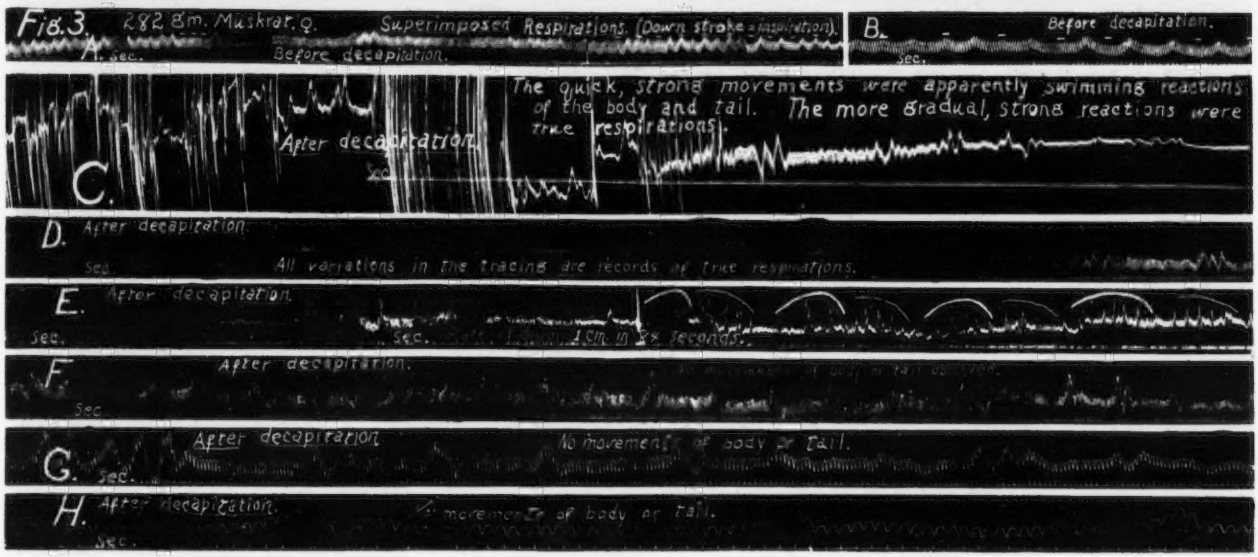


Fig. 2.
Blood pressure. Hg manometer.
Respirations (Down stroke = inspiration).
Mink. ♂. Cheyne-Stokes Breathing and Traube-Hering Waves.
Seconds and 100.00 mm Hg blood pressure line.

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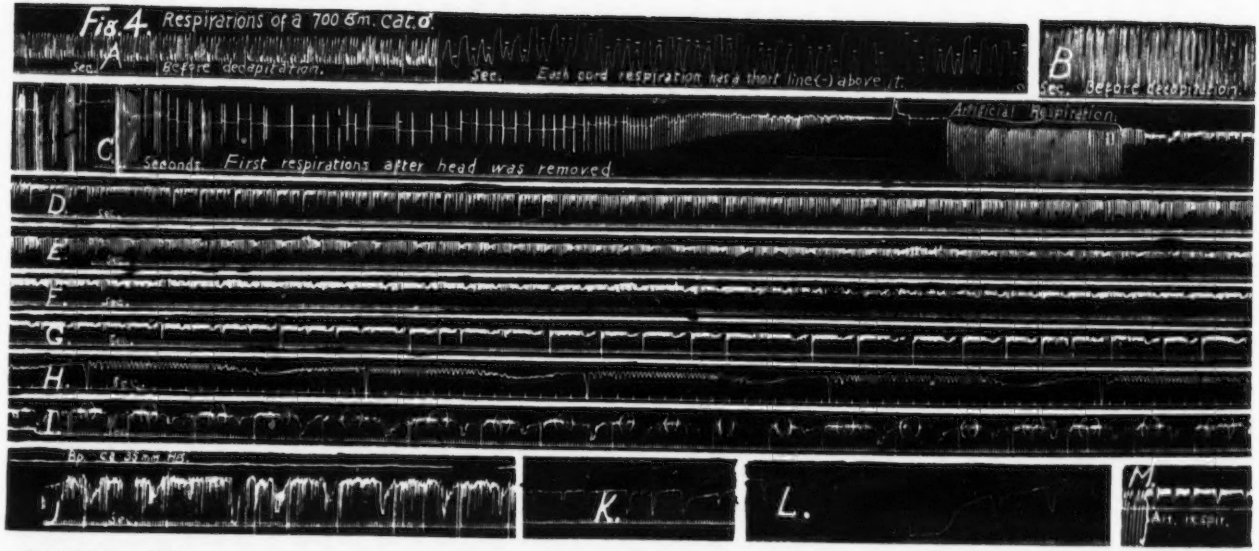


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considerable time. This is a reason for studying the respirations of a decapitated, non-aquatic mammal along with the breathing of the seal and other amphibious mammals. A further reason is that the strictly cord respirations of the decapitated cat are characteristically periodic or superimposed (fig. 4) and are very similar in some respects to the respirations of a normal aquatic mammal (fig. 3). Breathing of the normal aquatic mammal would seem, therefore, to be largely or entirely cord breathing. This idea is supported by the fact that the seal and muskrat breathe in very much the same way after as before decapitation (figs. 1 and 3).

The spinal cord of the mammal seems to contain many respiratory centers, for various kinds of breathing were obtained simultaneously or at separate times from the decapitated animals. The results lead to the conclusion that although the medulla possibly contains some true respiratory centers, its primary function is that of regulator. It normally regulates the action of the respiratory centers in the cord similarly as the cerebral centers regulate certain spinal reflexes. If one region of the medulla is disturbed, the cord reacts to cause gasping; if another region is disturbed, there is hyperpnea due to cord action, and so on. Likewise, disturbing the sciatic nerve alters the breathing, both before and after decapitation. Many of the impulses which reach the cord via the sciatic or certain neurones from the medulla merely influence the cord breathing. When the medulla is rendered anemic (as when the intracranial tension is increased intentionally or accidentally) or when its influence over the cord is partially or totally eliminated by any other means without greatly disturbing the cord, the uncontrolled reactions of the centers in the cord generally mean superimposed respirations, i.e., Cheyne-Stokes breathing of one variety or another. In very early life, presumably before the medulla has gained complete control over the respiratory centers in the cord, Cheyne-Stokes breathing is a frequent phenomenon. It seems probable that the medulla of the aquatic mammal gains only incomplete control over the respiratory centers in the cord. Due to the training which the aquatic mammal frequently receives, it continues to breathe in the Cheyne-Stokes fashion somewhat as it did in early life.

THE HIPPOPOTAMUS. The animal observed is a male, about fourteen years of age, in the Washington Park Zoo, Milwaukee. The animal inspires deeply just before submerging the nostrils. This inspiration may be of very long duration, continuing often for several minutes while the animal is under water. Either immediately or after several seconds, relatively rapid respiratory-like movements take place and are necessarily superimposed on the long inspiration. These under-water movements occur at the rate of fourteen or fifteen per minute. They appear to be abdominal primarily. The body of the animal sinks through a distance of

about 3 to 8 cm. each time the abdominal muscles contract and then rises through a corresponding distance when the same muscles relax. The animal may be likened, therefore, to the well-known toy known as the "diving devil." These bobbing movements of the animal's body evidently mean that the air in the greatly inflated lungs is compressed by the contraction of the abdomen and that the body accordingly sinks. A relaxation of the same muscles releases the pressure on the air in the lungs, and the body accordingly rises toward the surface. The body behaves in this way when no movements of the feet or head can be observed through the clear water. These abdominal movements are a form of under-water breathing in so far as they stir the air in the lungs and trachea and evidently enable the animal to utilize what would otherwise be dead air. Now superimposed on these rapid respirations is often a very rapid form of under-water movement. These are movements of the throat and various muscles of the face. Their usual rate is approximately 128 per minute. They were observed to occur at about half this rate in a very few instances. There is the possibility that these, also, are under-water respirations in the sense that they help to stir the air in the trachea and greatly inflated lungs. These movements do not cause the animal to rise and sink appreciably in the water, probably because they are not in phase with a vibration frequency of the huge body. An auxiliary respiratory mechanism which should not be overlooked is the heart. It is only reasonable to assume that the action of the heart mechanically stirs the air in the lungs and trachea, especially when the lungs are greatly distended as they are during the very deep, prolonged inspirations.

Aside from stirring the air in the lungs, the under-water movements, especially those of the abdomen, probably have useful effects on the circulation of the blood. Judging from the tracings obtained from the seal (fig. 1) and mink (fig. 2), the under-water respirations prevent a monotonous circulation by mechanically causing respiratory variations in it. These variations probably insure circulation of the blood to parts of the body at which there would be temporary stasis, relative or absolute, if the blood pressure varied with the individual heart beats only. Stasis would be all the more probable if the blood pressure should fall to a very low level as it tends to do sooner or later during a very long inspiration or during apnea (fig. 2). A second way in which the underwater movements may help the circulation is by mechanically keeping the pressure at a relatively high level, similarly as when the abdomen of an animal with a depressed circulation is massaged with the hand.

As the nostrils of the hippopotamus emerge from the water, a powerful expiration occurs. This is often followed by the deep inspiration which prepares the animal to submerge the nostrils. In some cases, especially when something attracts the animal's attention, several thoracico-

abdominal respirations may take place before the nostrils pass below the surface of the water. When the animal is in a dry stall, the three or four types of breathing occur simultaneously or separately. At least three of them ordinarily occur simultaneously when the animal is lying down. All of them are then effective respirations in the sense that they cause air to be drawn into and expelled from the lungs.

THE POLAR BEAR. Several animals of different ages and sexes were observed carefully in the Washington Park Zoo. All of the polar bears have access to large pools of water in which the animals are often completely immersed. The movements cannot be observed very well while the bears are under water. When out of the water, however, it is easy to observe that they frequently breathe in the Cheyne-Stokes fashion. A bear breathes at a relatively slow rate, depending somewhat on the size and general activity of the animal, and a very rapid form of abdominal breathing is periodically superimposed on the slower variety. Complete apnea of many seconds' duration alternating with considerably longer periods of breathing was observed on two of the larger bears while they were sitting or lying down.

It should be mentioned in connection with the polar bears that some of the bears of other varieties in the same garden also have access to pools of water but that these animals do not show the superimposed breathing. Their aquatic activities are limited to wading and drinking primarily. As is well-known, the non-amphibious bears often breathe periodically while in the state of hibernation, but this is conceivably due to the anemic condition of the medulla and the consequent partial or total loss of control of the higher centers contained therein over the true respiratory centers in the cord.

THE SEAL. Two adult, California sea lions, male and female, were observed in the Washington Park Zoo. The tracings of figure 1 were obtained in the Physiological Laboratory from a third adult sea lion which was captured near Santa Barbara, California, about forty-two days before the experiment was performed. Several days after it arrived in Milwaukee, the left eye began to protrude because of a tumor of the left optic nerve in the orbit. Soon thereafter, the animal refused food and became relatively lethargic. At the time it was anesthetized, the animal seemed at times to be in extreme agony and was at times in a semi-comatose state. The *post-mortem* examination revealed an infected tumor of the left optic nerve and incipient basal meningitis, the meninges at the base of the left cerebral hemisphere being inflamed. The breathing was not essentially different from that of the normal seals, this being an odd and almost unexplainable fact until we consider that the seal is normally a cord breather. The agony of the animal was inferred principally from

its periodic spells of howling. While in the state of ether anesthesia, the behavior was such as might be expected of a normal seal.

The experimental seal was anesthetized with ether, tracheotomized and placed in such a position on its back and right side that both front feet (flippers) could move freely without disturbing the position of the body. A thread was attached to the skin of the abdomen immediately below the last rib and sufficiently dorsal to the dorso-ventral line that it was not often struck by the end of the left flipper. The thread passed over a small pulley and then down to a loaded muscle lever which wrote on a smoked drum. After many respiratory tracings were obtained, the blood pressure was recorded from the left common carotid. The process of removing the head was begun after the seal had been anesthetized for about seven hours. (It appears to be desirable to keep any animal anesthetized for a few hours before decapitation.) The operation was done with the utmost care and required almost an hour. The last move in the operation was cutting the cervical cord several millimeters below the medulla oblongata. The breathing ceased completely while the vertebral vessels were being prepared for ligation. Artificial inflation of the lungs was started at this time, and the abdomen was massaged with the hand at frequent intervals. Spontaneous breathing was resumed about twenty-five minutes after the cord was cut.

*Figure 1, A.* A rapid Cheyne-Stokes is superimposed on a very slow Cheyne-Stokes. Only the first three elements, *1st*, *2nd* and *3rd*, of one respiratory paroxysm and the final element, encircled, of a preceding paroxysm of the extremely slow Cheyne-Stokes are shown. Five respirations of the cycle are omitted. Shortly after the beginning of a deep inspiration, the more rapid respirations become unusually rapid. The rapid respirations at the troughs of the slow waves are some typical under-water movements of the seal. They are largely abdominal and are comparable to the under-water, abdominal respirations of the hippopotamus. During the deep inspirations, the flippers often moved as they do in swimming. These movements of the flippers are evidently associated with the under-water breathing of the seal. The deep inspirations would probably have a different form if manifested while the animal is under water with its nostrils firmly closed instead of in the laboratory while a cannula is in the trachea. Under the laboratory conditions, each of the rapid respirations permitted more air to pass out of the lungs than into them. There was not a great volume of air to be expelled suddenly, therefore, shortly before the beginning of the succeeding deep inspiration. Instead, the chest and abdomen were greatly contracted long before time for the next deep inspiration. There are opportunities for such a slow Cheyne-Stokes to develop as a habit while the seal searches the bottom

of the ocean or hides from an enemy. The rapid Cheyne-Stokes in this tracing seems to be a more natural one, because seals frequently remain alternately above and below the surface of the water for periods ranging from about ten to twenty seconds.

As strange as it may sound, either the apneic periods or the periods of relatively slow breathing correspond to the respiratory activities of the normal animal prior to diving, i.e., between the time the nostrils appear above and disappear below the surface of the water. Before the occurrence of the under-water respirations of amphibious mammals was established, exactly the reverse of the actual process was predicted.

Much could be said concerning the special respiratory variations, the Traube-Hering waves, in the arterial blood pressure, but a thorough discussion of them would require considerable space and may well be undertaken in a separate paper. It may be remarked in passing that the Traube-Hering waves seem to be invariably caused by and are never the cause of the respiratory paroxysms. An examination of the blood-pressure curves permits a more accurate interpretation of the respiratory curves than would otherwise be possible because bodily movements, other than the respirations, disturbed the blood pressure very little or not at all. This is especially apparent in the latter part of *E* of figure 1 where certain extraneous movements of the body caused some irregularities in the respiratory but not in the blood pressure tracing. Attention may be called to the fact at this time that the Traube-Hering wave is essentially upward during the deepest and essentially downward in direction during the shallowest of the slow inspirations.

*Figure 1, B and B′.* Seals often play in the water somewhat as the porpoise does. They come to the surface regularly at intervals of four or five seconds. This activity should give rise to a relatively rapid Cheyne-Stokes, such as is indicated in *B*.

When the head of the anesthetized and tracheotomized seal was immersed in a bucket of water, the animal inspired very deeply and also very rapidly during the deep inspiration. This respiratory complex is shown in *B*. The very rapid movements were associated with movements of the same rate of the facial and neck muscles. The movements are difficult to interpret, but they correspond, probably, to the very rapid respirations of the hippopotamus. Such movements were obtained only twice. At other times, when the head was immersed, the reactions were like those shown in *B′*, i.e., they had a fairly close resemblance to the very deep inspirations in *A*.

The type of Cheyne-Stokes breathing shown in the latter part of *B′* is like that in the earlier part of *A*. The breathing indicated at the beginning of the tracing is different, however, from anything studied in *A* or *B*. The rapid expirations are active and the inspirations are relatively

passive. It may be observed that non-anesthetized seals sometimes breathe in the same way. It seems possible that these correspond to emergency, under-water respirations such as are shown by experienced human divers when they attempt to remain under water for an exceptionally long time. These movements can conceivably improve the circulation even if oxygen absorption at the lungs can no longer be facilitated by such reactions. It may be noticed that the active expirations changed to active inspirations when the animal inspired very deeply through the tracheal cannula.

*Figure 1, C.* A characteristic phenomenon shown in this series of superimposed respirations is the apparent breaking up of a slow wave into more rapid ones. The apparent fusion of two or more rapid waves to form a slower one is also shown. In many instances the respiratory paroxysms were observed to change suddenly to half or twice the usual rate. The more usual rate of the slow waves is pointed out in the tracing by the heavy, curved lines underneath the slow inspirations. The lighter, curved lines are underneath the inspirations of the more rapid waves which seemed to evolve from the slower ones. The rapid rate continued for only a short time in these instances. In other cases, long series of the relatively rapid paroxysms intervened between the slower ones. Such changes seem to mean that one form of slow breathing may give way suddenly to either a more rapid or a still slower form similarly as the dog, cat or human may suddenly change from one form of simple breathing to one of a different rate.

*Figure 1, D and E.* These two series of respirations show typical forms of breathing of the decapitated seal. The breathing was monotonously periodic during the approximate two hours the animal breathed after decapitation. The rapid respirations appear to be the two kinds shown in *B'*, i.e., active expirations followed by relatively passive inspirations alternated with relatively passive expirations followed by active inspirations. The impression was gained from observing the animal that the more quickly executed respirations were abdominal and that the less rapidly executed ones were thoracico-abdominal. This breathing was highly comparable, therefore, to the breathing of the kitten before decapitation (fig. 4, *A*). The decapitated muskrat was not such a disappointment, for it breathed in a greater number of ways (fig. 3).

THE MINK. An unusually fine specimen, male, was captured uninjured by means of a forked stick and the bare hands on a small tributary of the Milwaukee River. While the animal was held in the hands it ceased breathing many times. It was soon placed in a large money sack. No breathing could be detected at times through the thick cloth of the sack. When the sack was opened to examine the respirations, the eyes and general behavior of the animal showed that it was fully alive, and very rapid, shallow movements of the abdomen could be detected. The mink was

placed in a wire cage and kept in the laboratory for several hours for observation. When it lay quietly in a corner of its cage, superimposed breathing was easily perceived. When the cage was partially or totally immersed in a tank of water, the mink often remained under the water entirely motionless except for rapid, under-water movements which appeared to be respiratory-like reactions of the abdomen primarily. In a few instances the under-water movements seemed to be thoracico-abdominal. Bubbles never rose from the nostrils. The mink was finally placed in a small box with some cotton onto which ether could be dropped. Because of its extreme activity and viciousness, considerable difficulty was expected in anesthetizing the animal, but a small amount of ether caused it to lie in an apparently lifeless condition. Suddenly, however, it was in a fighting mood, but not until it was tied securely on its back. Periods of extreme activity alternated repeatedly with periods of relative inactivity during which there was only the heart beat and in some instances rapid movements of the abdomen as well. It was necessary to keep the animal in a rather profound state of anesthesia in order to secure respiratory and blood-pressure tracings. Some of the typical tracings are shown in figure 2. It is unfortunate that the means used to record the respirations were inadequate in certain respects.

*Figure 2.* Each respiratory paroxysm begins with a deep inspiration which is poorly shown in the figure because the respirations were recorded by means of a tambour attached by a rubber tube to a side arm of the ether bottle. This arrangement may be adequate to record relatively rapid respirations for certain purposes but quite inadequate to record very slow ones. If the inspiration is very slow, the tambour fills with air so rapidly from the ether bottle that the fall in the curve is much shallower and of shorter duration than it should be. Superimposed on the deep inspiration are some rapid thoracico-abdominal respirations which evidently correspond to the under-water, thoracico-abdominal movements of this animal. If this is the case, the ether slowed them down to almost one half the normal rate. Now a secondary Cheyne-Stokes breathing is superimposed on each of the long paroxysms. In other words, a rapid Cheyne-Stokes is superimposed on a slower Cheyne-Stokes. These two respiratory complexes can be traced to the animal's aquatic habits. When the mink merely plays in the water, it may appear at the surface and disappear in the water at a rapid rate. At other times, perhaps when it scents danger or is examining something in the water, it may remain submerged for a few minutes at a time and come to the surface for relatively brief intervals. When it remains under the water for a long time, its respiratory mechanism behaves somewhat as if the animal were coming frequently to the surface. The two varieties of respiratory complexes are shown well when the animal is in ether anesthesia.

There is no indication in the tracing of either a slow or sudden expiration corresponding to that which normally occurs when the animal emerges from the water. Neither was one observed. The equivalent of this expiration occurred, however, for the abdomen and chest became and remained appreciably smaller at some of the rapid respirations. This is the meaning of the upward variations in the curves over some of which white lines are drawn. This type of contraction of the abdomen and chest would probably not have occurred in the normal mink with firmly-closed nostrils under water. The strong expiration would have taken place at some point during the long apneic period indicated in the tracing.

As is shown in figure 2, the thoracico-abdominal respirations (the under-water type of movement) may have a profound effect on the blood pressure. The effect here amounts to 80 mm. Hg or more, but this is probably greater than it would be in ordinary life when there is no complete apnea preceding the deep inspiration. It must be borne in mind that the apneic period, as shown in the tracing, is normally filled with a powerful expiration, a number of thoracico-abdominal respirations somewhat slower than those of the under-water type and finally the deep inspiration which prepares the animal to submerge. When the mink was in the money sack, the period of greatest respiratory activity evidently corresponded to the apneic period in the tracing.

On *post-mortem* examination of this mink, five lung flukes were extracted from the lower lobe of the right lung. The other lobes of the lungs were in excellent condition. It seems quite improbable that these flukes altered the animal's reactions appreciably. The animal appeared to be entirely normal in all other respects.

THE MUSKRAT. Two adults, male and female, were observed carefully in their natural habitat on a tributary of the Milwaukee River. Three young muskrats, two males and one female ten or twelve weeks of age, were caught with steel traps and were observed and experimented on in the laboratory. Each of the males had a compound fracture of a hind leg, but their breathing was about the same as that of the female rat which was caught by the toes of a hind foot and scarcely injured.

Superimposed breathing was the rule with the muskrats, young and old, male and female. The captured rats were placed in glass aquaria with sufficient water for diving. The animals moved about all the time they were below the surface, and the under-water movements of the chest and abdomen could not be observed as well, therefore, as in the case of the mink or the two muskrats observed while at freedom. The Cheyne-Stokes breathing was very apparent during much of the time that the animals were quiet.

Series *A* to *H* inclusive, figure 3, are tracings which were obtained from the rat that was practically uninjured by the trap. It may be useful

to state that this rat (Fiber zibethicus L.) was a house builder. It reacted to the ether essentially as the cat or dog does. Some time after the baby muskrat was decapitated, unsuccessful efforts were made to obtain the blood pressure from a carotid artery. The arteries were too small and tender. An effort was then made to cannulate an iliac artery or the abdominal aorta, but opening the abdomen interfered seriously with the breathing and the animal soon died. The method used to record the respirations was essentially like that employed in the case of the seal. The muskrat, however, was placed directly on its back, and the thread from the muscle lever was attached to the tip of the ensiform cartilage. The respirations seemed to be the same when the ether was given by the drop method as when it was given with an ether bottle after tracheotomizing. Also, the respirations did not seem to differ in any interesting respect from those manifested before etherization.

*Figure 3, A.* Superimposed breathing is quite evident. The three short, straight lines drawn above an earlier part of the tracing indicate the falling out of three of the slow respirations. In some cases, not shown in the figure, the Cheyne-Stokes breathing ceased for a much longer time. In the latter part of the tracing, the short, white lines indicate the slow respirations that dropped out for a time and then reappeared suddenly. The general appearance of this part of the tracing indicates that two separate respiratory activities of the same slow rate may occur together at times with their individual elements alternating. Either one of these may cease or begin at any time. The temporary decrease in rate of the most rapid of the respirations at one point in the tracing could not be accounted for. At each end of the tracing the superimposed breathing is most evident. It is present but is not so evident between these two paroxysms. The most rapid respirations are usually more shallow between than during or close to the marked paroxysms of Cheyne-Stokes breathing. It may be noticed, also, that the Cheyne-Stokes paroxysms fall in the troughs of some extremely long respiratory waves which recurred with an astonishing periodicity.

*Figure 3, B.* The short, white lines drawn above the tracing indicate the alternate respirations of slow rate which gradually disappeared and later reappeared suddenly. Habitual Cheyne-Stokes breathers frequently react in this way.

*Figure 3, C.* About twenty minutes after the rat was decapitated and artificial respiration was started, the tail began to move from side to side. These movements were probably swimming reactions. They became gradually stronger until the entire body was involved. These movements of the body appeared to be effective respirations, for they kept the animal alive for many minutes after the artificial respiration was stopped. After a time the animal began to breathe easily and regularly between some of the

periods of strong bodily movement. This stage is shown in the earlier part of the tracing. Direct observation of the animal showed that the relatively slow variations of considerable amplitude were relatively powerful respirations which should not be confused with the apparent swimming reactions of the tail and body; the abdominal muscles contracted strongly and the chest expanded greatly at the same time or the abdominal muscles seemed to relax almost completely as the chest contracted. The diaphragm was not observed.

Near the end of the tracing, the most rapid of the respirations disappeared almost completely. This is a critical period in the life of any decapitated animal. The muskrat survived this period without assistance, but it is always necessary, it seems, to give the kitten artificial respiration at this point. Three kittens which were intentionally not helped at this period could not be revived a few minutes later while two others which were aided, as shown in figure 4, *C*, breathed for many hours. The seal was given artificial respiration during the critical period.

*Figure 3, D.* Excepting the presence of the relatively very powerful respirations, this tracing is essentially the same as *A* of this figure. Four types of breathing are superimposed.

*Figure 3, E.* The first part of this tracing corresponds to the highest general level of the tracing in *D*, and the paroxysm of Cheyne-Stokes breathing at the first of the tracing obtained on a very slow drum corresponds to the respiratory paroxysm at the end of *D*. The latter half of *E* shows the relatively very powerful respirations grouped more or less distinctly into paroxysms of Cheyne-Stokes breathing as is indicated by the curved lines drawn above the tracing. There can be observed a second type of Cheyne-Stokes breathing which occurs periodically and which is comparable to the Cheyne-Stokes paroxysms near the beginning and end of *D*.

*Figure 3, F, G and H.* These tracings were recorded in close succession with the drum moving at different, relatively rapid rates. These tracings afford opportunities to examine some of the different kinds of breathing more critically than can be done in the other tracings. It should be stated in connection with *F*, *G* and *H* that the exceptionally powerful respirations are not different from some which were recorded at two widely separate times from the animal before decapitation. They are, therefore, not characteristic of the decapitated muskrat except in so far as they occurred much more persistently after the head was removed. It is possible that these peculiar respirations result from some unobserved activity of the animal in the water or in its house. The remaining respiratory complexes of the muskrat appear to be subject to essentially the same explanations as were offered for similar complexes of the seal.

THE CAT. Five kittens, three females and two males, of about 700 grams

each, were the cats that breathed after decapitation. Three of the kittens, one female and two males, did not survive the critical period already mentioned in connection with the muskrat. The two kittens which survived this period were given artificial respiration at the time until there were some definite signs of spontaneous breathing. This period and the artificial respiration required for one of the kittens are shown in the latter part of *C* of figure 4. The tracings of figure 4 were taken from the ensiform cartilage as was done in recording the respirations of the muskrat. The down strokes mean inspirations.

*Figure 4, A and B.* Two kinds of breathing are shown in *A*. The larger elements indicate thoracico-abdominal and the smaller ones simple abdominal respirations. Assuming that the medulla had either never gained control over or had lost control over the one type of breathing already referred to as abdominal, these were called "cord respirations" and were singled out in the latter part of *A* by placing a short line above each element. *B* is an instance of some of the simple abdominal respirations being periodically sandwiched in between groups of thoracico-abdominal ones. This is a type of periodic breathing which is shown at times by normal, young animals. Such simple abdominal respirations seemed to predominate after decapitation.

*Figure 4, C, D, E, F, G and H.* These tracings were obtained in the order presented with not more than ten minutes elapsing between any two successive ones. After decapitation and many minutes of artificial respiration, the body of the kitten seemed to gasp. The artificial respiration was then discontinued. The gasping eventually developed into easy breathing which became gradually weaker until it practically disappeared. This was the critical period for the decapitated kitten. Respiratory paroxysms are quite apparent throughout the latter part of *D*. As can be seen in *E, F, G* and *H*, the breathing was exceptionally rapid during the troughs of the slow waves. The very rapid respirations were purely abdominal and were comparable to the under-water respiration of the seal and other amphibious mammals. In some instances the abdominal muscles almost writhed while the chest was in the inspiratory position. The new type of breathing which began in *G* consisted of strong thoracico-abdominal respirations.

*Figure 4, I, J, K, L and M.* A secondary Cheyne-Stokes developed and is pointed out by means of the parentheses in *I*. About an hour later, the breathing was very complex, as shown in *J*. Approximately five hours after *J* was recorded, the breathing was limited almost completely to the type of thoracico-abdominal reactions shown in *K*. Still later, as shown in *L*, there was no indication whatsoever of the rapid breathing. About forty minutes was required for the change from *K* to *L*. *L* was converted into *M* by giving artificial respiration for many minutes.

GENERAL DISCUSSION: The spinal cord contains nervous elements necessary for respiratory movements of the abdomen and chest, for the extensor-thrust and for various other reactions. We may profitably speak of spinal centers for such activities. Under normal conditions, the spinal centers are held in check by some groups of higher neurons which may be called regulating centers. All of the spinal reactions are profoundly affected when the flow of impulses between the cord and medulla oblongata is interrupted by cooling the upper cervical cord (Trendelenburg, 1910), by applying $\beta$-eucain locally at this region (Asher, 1902), by high cervical transection or by completely decapitating the animal. Until artificial respiration is given for some time following decapitation or certain substitutes for this operation, the only detectable reaction may be a feeble heart beat. It may again become possible, after a considerable time, to elicit the extensor-thrust, and spontaneous breathing might be resumed after some minutes or hours. It becomes eventually easier to elicit the extensor-thrust than before decapitation. The thrust is also stronger when elicited. Similarly, there is more breathing after decapitation; two or more kinds of breathing may occur simultaneously. It is largely because of the time elapsing between decapitation and the resumption of spontaneous breathing that we habitually speak of the vicarious action of the respiratory centers in the cord. However, consistent thinking leads to the absurd postulation of vicarious action of the nervous elements involved in the extensor-thrust if vicarious action of the respiratory centers in the cord is assumed.

Somewhat as Langendorff (1880), (1909) and Loeb (1903) have already advocated, the cord of the mammal, like that of some lower animals, seems to contain some true respiratory centers which are normally held in check (regulated) by some higher (regulating) centers that are located largely in the medulla. The term regulating center implies association neurons as well as two centers at different levels. Because of the association established between the groups of neurons at different levels, both centers of the pair function together. As long as the higher centers function one at a time (as higher nerve centers tend to do) there is action of only one spinal center at a time and only one kind of breathing is observed at a time. However, when the association neurons (in the upper cervical cord) are cut or incapacitated in certain other ways, the lower centers may react sooner or later as they tended to do in the early life of the animal before the associations were well established. There may also be a reversion to the infantile type of breathing (Cheyne-Stokes) when the medulla becomes anemic or when the regulating centers are affected in certain other ways. In all probability, it is a relatively very small number of adult animals and humans that live (without the aid of artificial respiration) to manifest Cheyne-Stokes breathing after the

regulating centers become unable to function. Artificial respiration must usually be given until some primitive associations between some peripheral neurons and the spinal centers are either reëstablished or merely strengthened. Ordinarily, therefore, considerable time should be expected to elapse before the respiratory centers in the cord are prepared to react in their apparently automatic way. This situation does not mean vicarious functioning of the spinal respiratory centers. It does mean that some elements of spinal nerves function in the place of some neurons in the upper cervical cord to excite the respiratory centers in the cord, but the functioning of even these neurons is not strictly vicarious because they functioned before as well as after the cord is cut. The impulses from these elements normally pass to higher centers, but when this becomes impossible they pass to centers in the cord, if to any at all. In case of the young or adult amphibious mammal and the young, non-amphibious mammal, the primitive associations are still strong enough that breathing will more probably be resumed after decapitation than in case of the adult, non-amphibious one in which the primitive or infantile associations have had ample time and opportunity to break down from disuse.

## SUMMARY

1. The usual activities of amphibious mammals are such that separate respiratory centers in the cord continue (in adult life) to function relatively unrestrained by certain higher centers (regulating centers).

2. The amphibious mammals keep in training to manifest *certain* kinds of respiratory complexes (Cheyne-Stokes breathing).

3. Excepting the phenomenon of respiratory shock, removing the higher centers (by decapitating) scarcely altered the breathing of the muskrat, and the effect on the sea lion was not very great.

4. The amphibious mammals breathed both before and after decapitation somewhat as the kitten did after its head was removed.

5. The functioning of the respiratory centers in the mammalian cord is not vicarious.

6. With the possible exception of the tracings from the muskrat, the results do not exclude the possibility or even minimize the urge to believe that some true respiratory centers are in the brain stem and perhaps also in the cerebrum.

Of the several people to whom I feel indebted for assistance of some sort in this investigation, I wish to express my appreciation especially to Mr. Edward H. Bean, Director of the Washington Park Zoo, to Dr. Eben J. Carey of the Anatomy Department and to Mr. Eugene H. Payne of the Physiology Department.

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# THE EFFECT OF THE LUXUS CONSUMPTION OF MEAT UPON THE VOLUNTARY ACTIVITY AND GROWTH OF THE ALBINO RAT[1]

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The minimum protein requirement for man as well as for many of the lower animals has been fairly well established, but what constitutes the optimal level of intake is a question upon which we have little convincing evidence. The works of Neuman (1902), Chittenden (1904), Fisher (1907) and Hindhede (1913) show that men can maintain themselves for extended periods of time in good health and without loss of weight on a level of protein intake well below that of the average man. They do not show, however, that this low level is the optimal. The work of Chalmer Watson (1906), who showed that rats failed to thrive on an exclusively flesh diet, is of little value since the discovery of accessory substances, for vitamin deficiency was undoubtedly a large factor in causing his results. A similar criticism holds for much of the older work.

Starling (1919) has reported an instance of increased efficiency of laborers following a more liberal allowance of protein in their ration. The same author states (1920) that "it has been found that for men leading a sedentary life a moderate protein and fat diet is more suitable than one consisting mostly of carbohydate." He bases this conclusion upon the fact that with many men the carbohydrate tolerance is much lower than that for protein and presumably this tolerance would be decreased by excessive consumption of carbohydrate. Howell (1925) thinks that the optimal level of protein intake is probably well above the minimal requirement, "since our instinctive appetite seems to lead us to seek the protein diet." Slonaker (1912) has reported that rats upon a diet containing lean meat as its protein ingredient grew more rapidly, were more active and lived longer than those on a purely vegetable diet. The quantitative factors in his work, however, were not well controlled and it is possible that his rats on a vegetable diet were not receiving an adequate amount of complete protein.

We have under way in this laboratory experiments by means of which

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we hope to establish the optimal level of protein intake for the white rat. The first phase of this work, which deals with the effect of adding a liberal quantity of meat to a diet already adequate, is herein reported.

Fifty-six females and thirty-five males were used in the experiments. All of these animals were placed in cages similar to that described by Stewart (1898). Each cage consists of a small retiring compartment $4 \times 5 \times 6$ inches in dimension in which the animals received their food and water and in which they spent a considerable proportion of their time in a quiet state. Communicating with the retiring cage is a revolving wheel one foot in diameter and six inches in cross section. This is made of two metal discs, with hardware cloth serving as a treadway between them. Attached to the axle of the cylinder is a Veeder counter of the ratchet type which records additively the revolutions, irrespective of direction. A complete description together with a photograph of the cage has been published by Durrant (1925).

As discussed in other contributions from this laboratory, the variability of activity, even among litter mates, is surprisingly great (Gans and Hoskins, 1926). For this reason, in all experiments the animals were first typed, that is, a group was put in the wheels for a period of a month and then classified as regards spontaneous activity. In making up the experimental groups the most active animal was taken as a control, the next as a meat-fed animal, the next as a control, and so on throughout the series. In this way the control group consisted of animals which, in the beginning, averaged slightly higher activity than the meat-fed animals. It was not deemed worth while to maintain litter controls since the variation within litters was found to be quite as great as that between individuals of different litters.

During typing all animals were placed on a diet consisting of whole wheat flour 62.5 per cent, dry casein 15 per cent, skim milk powder (analac) 15 per cent, $CaCO_3$ 1.5 per cent, NaCl 1 per cent and cotton-seed oil 5 per cent. About 200 cc. of cod liver oil were added to each hundred pounds of this food. This is substantially the diet recommended by McCollum. Using the analysis of whole wheat flour given by Atwater and Bryant (1906) and the approximate analysis of the skim milk powder provided by the makers, we computed that this food contained approximately 27 per cent protein, 53 per cent carbohydrate, 6 per cent fat, 4 per cent mineral matter and 10 per cent water. From these figures it follows from calculations that 100 grams of this food represent 382 calories, protein furnishing 29 per cent of the total, carbohydrate 57 per cent and fats 14 per cent. This food is made up in dry cakes and is kept before the animals at all times. There is, of course, a certain amount of variation from time to time in the moisture content of the food. Determinations made on samples that had been kept for varying lengths of time showed, however, that this

variation was rarely more than 1 per cent. One set of determinations made after three days of continuous rain, showed an increase in moisture

TABLE 1A

*Average daily activity of female rats in revolutions. The revolving cylinders are one foot in diameter*

| RAT NUMBER | JANUARY | FEBRUARY | MARCH | APRIL | MAY | JUNE | JULY | AUGUST | SEPTEMBER | OCTOBER | NOVEMBER | DECEMBER |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Meat fed** | | | | | | | | | | | | |
| 732 | 12169 | 14618 | 14432 | 9868 | 5793 | 6117 | 9349 | 9907 | 9391 | 12060 | 8116 | 8067 |
| 829 | 1693 | 15773 | 7958 | 4455 | 1552 | 1210 | 1165 | 780 | 1033 | 618 | 1764 | 2268 |
| 741 | 8999 | 11096 | 7828 | 7776 | 8664 | 7231 | 9327 | 8628 | 4526 | 4164 | 5267 | 5122 |
| 727 | 10960 | 14574 | 10342 | 8897 | 6163 | 7220 | 5707 | 3771 | 2600 | 3447 | 2363 | 1011 |
| 730 | 10375 | 14379 | 16727 | 10212 | 10763 | 8226 | 8382 | 6410 | 7475 | 6288 | 7095 | 5430 |
| 750 | 5957 | 6914 | 12549 | 4559 | 1239 | 5055 | 4128 | 2628 | 2497 | 1375 | 919 | 250 |
| 751 | 8708 | 10991 | 6939 | 5639 | 4503 | 3226 | 2789 | 4535 | 2875 | 3048 | 4157 | *1734 |
| 835 | 1920 | 9711 | 12282 | 9478 | 8214 | 5104 | 2400 | 2232 | 3947 | 5216 | 3106 | 2834 |
| 791 | 8529 | 13092 | 14695 | 5171 | 6818 | 5208 | 2452 | 6384 | 6477 | 4579 | 3275 | 3762 |
| 798 | 8274 | 9631 | 8078 | 6502 | 7281 | 14206 | 12409 | 15123 | 13882 | 10714 | 2691 | 2138 |
| 796 | 1324 | 3353 | 3173 | 8016 | 6207 | 5926 | 5451 | 5713 | 5105 | 4122 | 2989 | 211 |
| 842 | 745 | 5901 | 6362 | 4785 | 6085 | 6119 | 2364 | 567 | 2764 | 1324 | 2102 | 1965 |
| 818 | 1100 | 7533 | 10440 | 4255 | 4390 | 3605 | 2985 | 4173 | 1673 | 1976 | 1255 | 1631 |
| Avg. | 6212 | 10582 | 10139 | 6893 | 5975 | 6035 | 5301 | 5450 | 4942 | 4533 | 3469 | 2802 |
| **Control** | | | | | | | | | | | | |
| 728 | 14335 | 18089 | 11836 | 8196 | 9425 | 5009 | 3927 | 5007 | 4587 | 1117 | 743 | 887 |
| 738 | 7640 | 11701 | 9078 | 7975 | 10719 | 7680 | 8977 | 10857 | 9228 | 9581 | 6180 | 5145 |
| 739 | 13347 | 11489 | 7408 | 7012 | 7790 | 8177 | 8288 | 8866 | 7025 | 5694 | 2775 | 3075 |
| 731 | 13081 | 12494 | 10648 | 7562 | 5596 | 6717 | 6066 | 4032 | 3559 | 3348 | 3621 | 3751 |
| 832 | 1698 | 4523 | 7791 | 8447 | 7677 | 7970 | 5412 | 8055 | 3826 | 4201 | 2513 | 2076 |
| 833 | 1623 | 7661 | 10208 | 11287 | 10969 | 17045 | 15689 | 16767 | 17483 | 12332 | 9171 | 11141 |
| 836 | 896 | 9499 | 9096 | 6810 | 4867 | 1313 | 2502 | 2742 | 2425 | 1777 | 1745 | 1937 |
| 837 | 712 | 6750 | 10031 | 10020 | 9395 | 7805 | 7543 | 7150 | 5651 | 1609 | 3623 | 5692 |
| 792 | 9742 | 13679 | 11930 | 5059 | 6603 | 9615 | 7898 | 11343 | 9090 | 8713 | 7838 | 10449 |
| 797 | 922 | 9706 | 9847 | 9897 | 6531 | 9251 | 8143 | 5981 | 4966 | 4841 | 978 | *10 |
| 806 | 5777 | 14397 | 6068 | 5306 | 8972 | 9138 | 9562 | 14792 | 11439 | 6386 | 4113 | 5955 |
| 841 | 1338 | 5939 | 10290 | 8700 | 5938 | 12436 | 11656 | 9032 | 11833 | 8904 | 7390 | 11581 |
| 843 | 875 | 4305 | 6643 | 5091 | 3064 | 4415 | 2926 | 4118 | 4364 | 3455 | 4896 | 5731 |
| 816 | 1344 | 7987 | 8415 | 6146 | 8079 | 6011 | 5682 | 4423 | 3756 | 4140 | 4880 | 6620 |
| Avg. | 5238 | 9873 | 9235 | 7679 | 7545 | 8042 | 7448 | 8083 | 7088 | 5436 | 4319 | 5219 |

*Rat died.

content of slightly more than 2 per cent. The average of all moisture determinations was a little more than 10 per cent. This figure is therefore used in all calculations. Our experience has shown that rats thrive

admirably on this diet. In addition to this standard food the animals were kept almost continually supplied with dry yellow corn and were fed once a week all the cabbage or lettuce they would eat.

After the arrangement of the animals in two groups, as explained above, to the stock diet of one lot were added daily about 8 grams of fresh lean meat cooked rare. This meat was eagerly eaten. Again using Atwater and Bryant's analysis, this amount of meat would represent 2.25 grams of protein and about 0.8 gram of fat, corresponding to a food value of about 17 calories. During the first ten months of the experiment no effort was

TABLE 1B

*Average daily activity of male rats in revolutions. The revolving cylinders are one foot in diameter*

| RAT NUMBER | JANUARY | FEBRUARY | MARCH | APRIL | MAY | JUNE | JULY | AUGUST | SEPTEMBER | OCTOBER | NOVEMBER | DECEMBER |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Meat fed** | | | | | | | | | | | | |
| 800 | 3091 | 5734 | 4899 | 2426 | 3050 | 2643 | 2795 | 2097 | 1868 | 1196 | 1763 | 1390 |
| 801 | 4125 | 7556 | 6107 | 7506 | 6951 | 3825 | 3739 | 3341 | 3007 | 1085 | 1671 | 1728 |
| 822 | 2392 | 7744 | 5950 | 1223 | 853 | 956 | 919 | 1500 | 686 | 906 | 793 | 128 |
| 825 | 2312 | 2301 | 6095 | 4096 | 5085 | 1325 | 1430 | 1969 | 1248 | 1015 | 650 | 561 |
| 827 | 1547 | 5051 | 11459 | 12355 | 11565 | 10129 | 6275 | 10687 | 12512 | 6461 | 4849 | 5617 |
| Avg. | 2693 | 5677 | 6902 | 5521 | 5501 | 3775 | 3032 | 3919 | 3864 | 2133 | 1945 | 1885 |
| **Control** | | | | | | | | | | | | |
| 776 | 3334 | 3262 | 2738 | 2664 | 3559 | 1854 | 1853 | 1326 | 1456 | 1357 | 1290 | 1350 |
| 790 | 3700 | 4059 | 5602 | 5156 | 5524 | 5111 | 2948 | 3523 | 4279 | 3779 | 2984 | 2472 |
| 803 | 1046 | 5173 | 4723 | 4610 | 2655 | 2839 | 1593 | 1116 | 714 | 381 | 1687 | 143 |
| 760 | 4771 | 10201 | 7013 | 7756 | 6747 | 6023 | 6766 | 5481 | 1277 | 1206 | 1540 | 1041 |
| 787a | 2240 | 7123 | 3408 | 2419 | 2832 | 1669 | 1242 | 1448 | 1377 | 1557 | 1815 | 1417 |
| 775 | 3346 | 7520 | 7745 | 6915 | 6313 | 7595 | 7361 | 8271 | 6668 | 2598 | 2601 | 3129 |
| Avg. | 3073 | 6223 | 5205 | 4920 | 4605 | 4182 | 3627 | 3528 | 2629 | 1813 | 1986 | 1592 |

made to measure the exact food consumption. However, during November and December the food consumed was carefully weighed daily, with an accuracy of plus or minus one gram. From the figures obtained in this way it was determined that the control females consumed approximately 13.8 grams and the control males 15 grams of the stock ration per day. The meat-fed females consumed 11.4 grams and the meat-fed males 13.4 grams of the stock food each day in addition to their 8 grams of meat. This means that the total intake of the meat-fed females was 61 calories per day and of the meat-fed males 67 calories per day, as against 53 calories for the control females and 57 calories for the control males. The feeding

of corn was discontinued during this period. Cabbage was fed as usual but the caloric content of the small quantity eaten was so low as to be negligible. The increased food consumption of the meat-fed animals can

TABLE 2A

*Weights in grams of female rats, according to age*

| RAT NUMBER | AGE IN DAYS | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 30 | 60 | 90 | 120 | 150 | 180 | 210 | 240 | 270 | 300 | 330 | 360 | 390 |
| **Meat fed** | | | | | | | | | | | | | |
| | *grams* | *grams* | *grams* | *grams* | *grams* | *grams* | *grams* | *grams* | *grams* | *grams* | *grams* | *grams* | *grams* |
| 732 | 60 | 135 | 182 | 210 | 240 | 255 | 270 | 280 | 248 | 267 | 271 | 273 | 281 |
| 829 | 45 | 128 | 167 | 198 | 217 | 242 | 242 | 258 | 249 | 250 | 253 | 255 | 258 |
| 741 | 60 | 107 | 181 | 221 | 255 | 264 | 289 | 318 | 288 | 315 | 330 | 338 | 320 |
| 727 | 50 | 93 | 157 | 185 | 229 | 233 | 263 | 268 | 267 | 258 | 261 | 264 | 261 |
| 730 | 55 | 100 | 157 | 203 | 233 | 244 | 265 | 266 | 272 | 270 | 270 | 267 | 264 |
| 750 | 47 | 99 | 147 | 183 | 210 | 228 | 243 | 222 | 232 | 234 | 243 | 233 | 226 |
| 751 | 39 | 96 | 144 | 174 | 207 | 234 | 248 | 265 | 274 | 250 | 264 | 276 | 258 |
| 835 | 30 | 105 | 152 | 173 | 183 | 197 | 189 | 186 | 204 | 201 | 205 | 210 | 207 |
| 791 | 40 | 102 | 141 | 175 | 228 | 255 | 255 | 231 | 270 | 266 | 273 | 267 | 276 |
| 798 | 25 | 114 | 157 | 192 | 217 | 210 | 222 | 230 | 225 | 230 | 234 | 249 | 251 |
| 842 | 28 | 120 | 168 | 195 | 222 | 243 | 251 | 246 | 255 | 262 | 261 | 252 | 253 |
| 818 | 25 | 92 | 170 | 205 | 219 | 230 | 240 | 246 | 279 | 276 | 288 | 288 | 287 |
| 796 | 40 | 70 | 157 | 204 | 234 | 242 | 250 | 250 | 261 | 256 | 251 | 262 | 264 |
| Avg. | 41.8 | 104.7 | 160.0 | 193.7 | 222.6 | 236.7 | 248.2 | 251.2 | 255.7 | 256.5 | 261.8 | 264.2 | 262.0 |
| **Control** | | | | | | | | | | | | | |
| 728 | 57 | 95 | 159 | 180 | 201 | 207 | 218 | 225 | 232 | 225 | 215 | 209 | 214 |
| 738 | 70 | 90 | 152 | 189 | 205 | 213 | 216 | 240 | 232 | 222 | 216 | 207 | 220 |
| 739 | 70 | 110 | 157 | 176 | 183 | 186 | 198 | 204 | 207 | 210 | 208 | 213 | 217 |
| 731 | 70 | 117 | 172 | 177 | 196 | 186 | 225 | 246 | 240 | 241 | 246 | 245 | 243 |
| 832 | 33 | 98 | 150 | 165 | 175 | 195 | 206 | 216 | 225 | 202 | 211 | 210 | 216 |
| 833 | 35 | 82 | 123 | 143 | 150 | 159 | 169 | 176 | 174 | 177 | 176 | 183 | 180 |
| 836 | 35 | 120 | 198 | 211 | 238 | 231 | 225 | 224 | 223 | 219 | 232 | 232 | 233 |
| 837 | 33 | 105 | 158 | 175 | 190 | 195 | 195 | 197 | 196 | 192 | 198 | 210 | 209 |
| 792 | 42 | 105 | 144 | 150 | 177 | 196 | 216 | 223 | 223 | 225 | 220 | 241 | 235 |
| 797 | 41 | 111 | 170 | 212 | 232 | 235 | 240 | 246 | 244 | 238 | 225 | *210 | |
| 806 | 40 | 129 | 166 | 186 | 202 | 204 | 216 | 213 | 212 | 211 | 195 | 221 | 219 |
| 841 | 35 | 129 | 186 | 201 | 228 | 225 | 235 | 243 | 241 | 235 | 232 | 238 | 250 |
| 843 | 40 | 123 | 172 | 191 | 226 | 226 | 249 | 234 | 229 | 234 | 227 | 240 | 235 |
| 816 | 35 | 120 | 173 | 182 | 198 | 215 | 234 | 231 | 231 | 238 | 242 | 245 | 235 |
| Avg. | 45.4 | 109.6 | 162.9 | 181.3 | 200.1 | 205.2 | 217.3 | 222.7 | 222.1 | 219.2 | 217.4 | 221.7 | 223.5 |

* Rat died.

be correlated with the fact that they averaged nearly 20 per cent greater body weight than the control animals. The protein in the diet of the meat-fed group represented about 36 per cent of the total energy intake, while

with the control animals the protein furnished about 29 per cent of the total calories. Determination by Folin's micro-method (1922) of the total nitrogen excreted in the urine on the second and third days of a three day fast showed that the meat-fed males excreted about 1.21 mgm. of nitrogen per gram of body weight, while the controls in the same time and under comparable conditions excreted only 1.08 mgm. per gram of body weight. In the case of the females there was but little difference between the two groups. The meat-fed animals excreted 1.06 mgm. per gram of body weight while for the controls the corresponding figure was 1.03 mgm. It seems probable, therefore, that a higher level of nitrogen storage was

TABLE 2B

*Weights in grams of male rats, according to age*

| RAT NUMBER | AGE IN DAYS | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 30 | 60 | 90 | 120 | 150 | 180 | 210 | 240 | 270 | 300 | 330 | 360 | 390 |
| **Meat fed** | | | | | | | | | | | | | |
| | *grams* | *grams* | *grams* | *grams* | *grams* | *grams* | *grams* | *grams* | *grams* | *grams* | *grams* | *grams* | *grams* |
| 800 | 35 | 126 | 245 | 312 | 341 | 368 | 381 | 385 | 398 | 403 | 394 | 398 | 402 |
| 801 | 35 | 136 | 236 | 290 | 305 | 317 | 354 | 355 | 366 | 384 | 366 | 377 | 386 |
| 822 | 64 | 181 | 278 | 316 | 321 | 362 | 364 | 379 | 368 | 377 | 384 | 391 | 392 |
| 825 | 61 | 197 | 308 | 318 | 365 | 416 | 429 | 458 | 468 | 456 | 488 | 470 | 485 |
| 827 | 49 | 185 | 210 | 254 | 258 | 276 | 285 | 286 | 288 | 300 | 312 | 312 | 329 |
| Avg. | 48.8 | 165.0 | 255.4 | 298.0 | 318.0 | 347.8 | 362.6 | 372.6 | 377.6 | 384.0 | 388.8 | 389.6 | 398.8 |
| **Control** | | | | | | | | | | | | | |
| 776 | 45 | 132 | 254 | 320 | 350 | 380 | 400 | 411 | 450 | 427 | 429 | 442 | 451 |
| 790 | 42 | 130 | 228 | 262 | 268 | 285 | 303 | 320 | 321 | 316 | 310 | 309 | 328 |
| 803 | 40 | 138 | 192 | 228 | 251 | 268 | 280 | 276 | 276 | 256 | 260 | 260 | 246 |
| 760 | 47 | 68 | 178 | 204 | 218 | 239 | 250 | 260 | 274 | 268 | 244 | 245 | 234 |
| 787a | 47 | 130 | 202 | 225 | 258 | 284 | 296 | 298 | 326 | 332 | 290 | 292 | 308 |
| 775 | 45 | 140 | 270 | 317 | 333 | 350 | 364 | 372 | 363 | 356 | 372 | 365 | 368 |
| Avg. | 44.3 | 123.0 | 220.7 | 259.3 | 279.7 | 301.0 | 315.5 | 322.8 | 335.0 | 325.8 | 317.5 | 318.8 | 322.5 |

obtained in the meat-fed than in the control animals. It is planned to give a complete report on this phase of the problem in a later paper.

The revolving cage counters were read once a day, at noon, and all animals were weighed once a week. The activity was averaged for five day periods, and both activity and body weight plotted on the same graph, a separate graph being kept for each animal.

At the beginning of the experiment there were twenty-eight females and eighteen males in the meat-fed series, and an equal number in the control series. The females were arranged originally in four groups. Group 1 consisted of ten meat-fed and ten control animals. The meat feeding in

this group was begun January 16, 1925. Group 2 consisted of six meat-fed and six control animals. The meat feeding of this group was begun February 11, 1925. Group 3 contained seven meat-fed and seven control animals and meat feeding was begun February 18; while group 4 consisted of five meat-fed and five control animals and the meat feeding was begun with this final group on March 24. The males were all arranged in a single group and received their first allowance of meat on February 18.

In group 1 of the females the meat-fed rats showed a decided advantage in activity for the first two and a half months of meat feeding. However, no such advantage was evident in any of the other groups. In order to determine whether or not this higher activity on the part of the meat-fed animals was in any way a result of the diet, both meat-fed animals and their controls were subjected to a five day fast, after which the control animals were fed meat while the animals that had previously been receiving meat were now used as controls. The fast period served a double purpose. First it was designed to exhaust the stored nitrogen in the animals' bodies, thus putting both the controls and the meat-fed animals on an equal basis. In the second place it made possible a comparison of the resistance to starvation exhibited by meat-fed and control animals. Three of the meat-fed animals died during the fast while all of the controls survived. Since two meat-fed animals and two controls had been accidentally killed before the beginning of the fast the numbers in this group were reduced to five animals that had received meat previous to starvation and were used as controls following it and seven animals that were treated in a reverse manner. The activity of the two groups averaged for five day periods for fifty days preceding the period of starvation and for the same length of time following it is shown in figure 1. Inspection of this graph shows that there was little or no change in the relative activity of the two groups following the fast and reversal of meat feeding. Both groups show a considerable drop in activity after the period of starvation. The animals that were originally receiving meat averaged about 6,800 revolutions per day preceding the fast and about 5,200 following it. This is a drop of about 22 per cent. On the other hand, the animals that were originally used as controls and received meat following starvation dropped but 7 per cent in activity, that is, from 4,700 to 4,400 revolutions per day. This therefore indicates that meat feeding was slightly advantageous. This fact is not borne out however on a similar experiment which was carried out on males.

Seven meat-fed males and their controls were subjected to the procedure just described for the females. One meat-fed and one control male died during the period of starvation, which left six animals in each group to be considered in the final analysis of data. In this case the meat-fed animals showed an activity lower than their controls preceding the period of star-

vation. Figure 1 shows the activity of both groups of males preceding and following the period of starvation. The animals which received meat previous to the fast averaged 3,700 revolutions per day before the fast and 2,800 after. This is a drop of 24 per cent. The animals that were fed meat following the fast dropped from 5,400 to 3,800 revolutions per day, which is slightly more than a 30 per cent decrease. We must conclude, therefore, that meat feeding had practically no effect upon the activity of the males in this group, even though there seemed to be a slight beneficial result to the females.

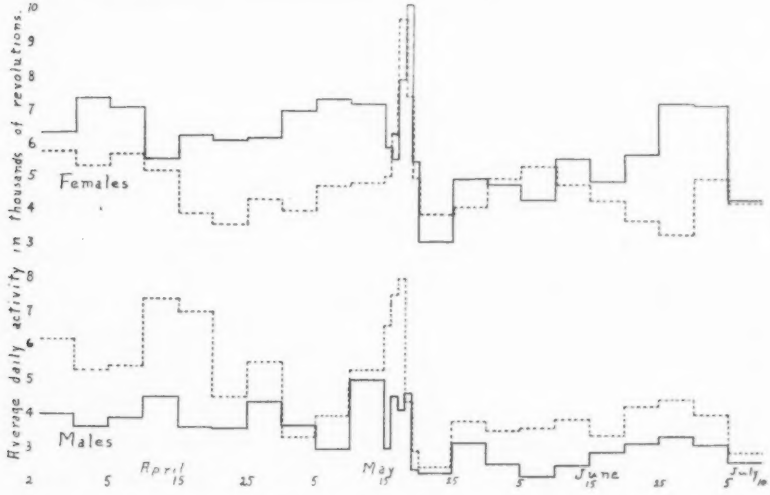


Fig. 1. Graphic representation of the average daily activity of thirteen female and twelve male rats before and after a five-day fast. The broken line shows the activity of animals that received meat in addition to their regular ration following the fast, while the continuous line represents the activity of animals that were fed meat before the fast but not after it.

The females of the remaining three groups and the rest of the males were continued according to their original distribution as meat-fed or control animals, throughout the entire experiment. They have been under observation now for more than a year. The composite graphs for both males and females showing the relative activity of meat-fed animals and their controls is given in figure 2. From inspection of these graphs it is evident that the average activity of the meat-fed females dropped off considerably more rapidly than that of their controls, while among the males the meat-fed group showed a slightly slower falling off of activity than did their controls. Indeed the meat-fed males had a decided advantage most of the

time. In only three months was their activity lower than that of their controls. A study of the individual activity curves, however, shows that in only five cases among the females and four among the males did a meat-fed animal show an increase in activity over its control following the feeding of meat, while in the case of four females and one male the activity of the meat-fed animal fell off more rapidly than that of the control. In none of the other animals was there any significant difference between the activity of the meat-fed animal and its control. The only conclusion that can be drawn, therefore, is that the addition of meat to a diet already con-

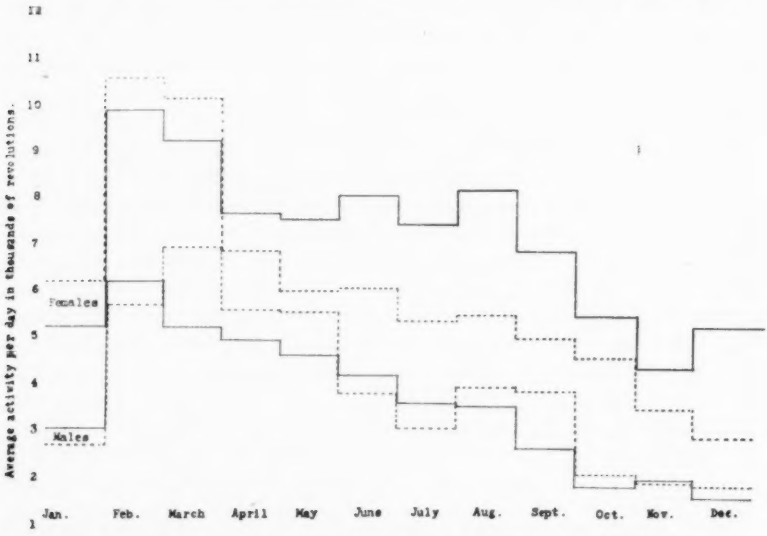


Fig. 2. Relative activity of control and meat-fed rats over a period of one year. The broken line indicates meat-fed animals and the continuous line controls. The male and female animals have been plotted separately. The revolving cylinders are one foot in diameter.

taining an adequate amount of protein leads to no consistent or significant effect upon the activity.

Of a much more positive nature, however, are the results obtained on body weight. Counting only the animals that have survived the entire experiment, there is only one female and one male in which the weight advantage does not lie with the meat-fed animal. The average weight of the meat-fed group was approximately 20 per cent greater at maturity than that of the controls. The data on body weight are tabulated in table 2, and shown graphically in figure 3. An inspection of these graphs shows clearly that both in regard to rate of growth and body weight at maturity

the advantage lay clearly with the meat-fed groups. A similar advantage of meat feeding is seem in the recovery of body weight following starvation. Figure 4 shows the loss of weight of the animals starved in the first experiment discussed in this paper and their subsequent recovery. In order to show the results more clearly the weights are plotted as percentage of the original average body weight of the animals. Males and females are averaged together in this graph. The animals that received meat previous to the period of starvation lost about 20 per cent of their body weight, while those that had not received meat lost about 22 per cent. The recovery was much more rapid for the meat-fed animals than for their con-

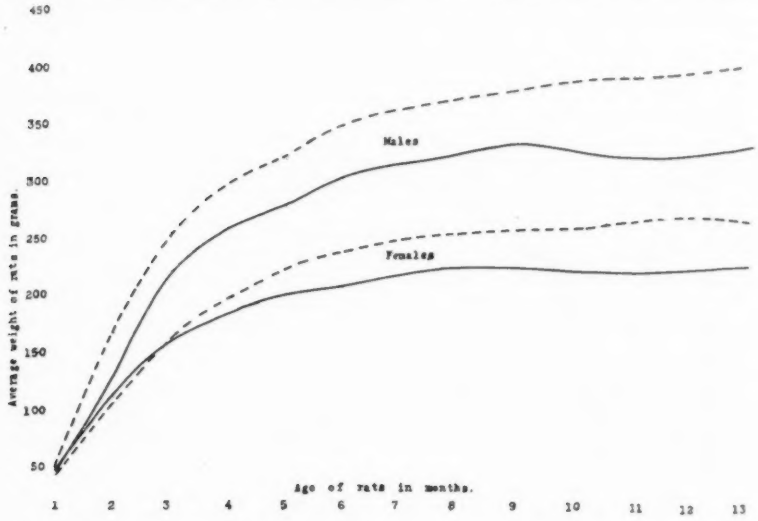


Fig. 3. Growth curves of meat-fed and control rats. Broken line meat-fed, continuous line control rats. Males and females have been plotted separately.

trols. Nine days after the end of the fast the meat-fed animals were back to their original weight, whereas it required fifteen days for the control animals to reach the 100 per cent line. Forty days after the end of the fast period the animals which were being fed meat weighed 15 per cent more than they had at the beginning of the fast. The control animals, on the other hand, weighed at this time only about 5 per cent more than their original weight.

The fact has already been mentioned that several of the animals did not survive for the entire duration of the experiment. In all, seventeen females and eight males died. Six of these females were accidentally killed early in May and therefore should not be considered. Three meat-fed

females and one meat-fed and one control male died during the five day fast that has already been discussed. This leaves eight females and six males that died of unknown cause. Of these, six of the females and five of the males belonged to the meat-fed series. The significance, if any, of these figures has not as yet been determined, but further experiments are now in progress by means of which it is hoped to determine the effect of the luxus consumption of meat upon life span.

It would be unwise to generalize from a single set of experiments which involved such a relatively small number of animals. However the data

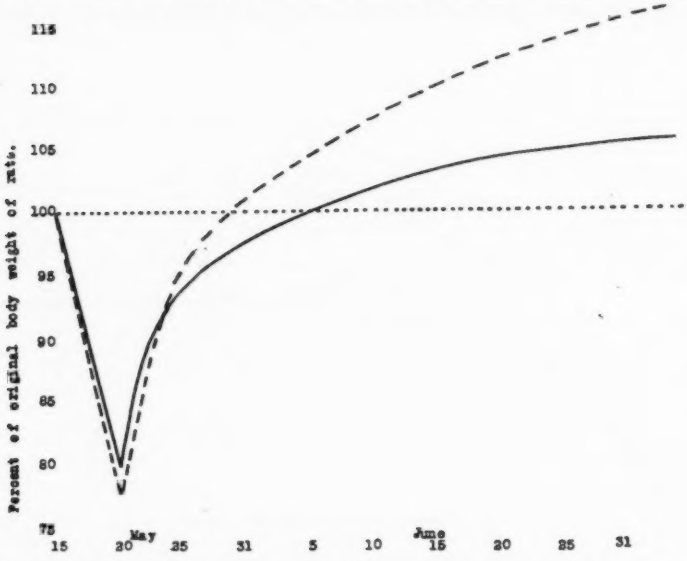


Fig. 4. Graph showing the recovery from starvation of meat-fed and control rats. The continuous line represents the average variation in body weight of eleven rats that received meat previous to the five day period of starvation and were used as controls following it. The broken line shows the average for thirteen rats that were used as controls previous to the fast period and received meat daily following it. The fast was from May 15 to 20.

reported here on growth and the recovery from starvation would seem to justify, at least tentatively, the conclusion that the luxus consumption of meat is beneficial wherever growth processes are involved.

## SUMMARY

The addition of 8 grams of meat daily to the diet of rats already receiving a ration containing approximately 28 per cent of protein, mostly casein, had the following effects.

1. The voluntary activity was changed but little and this not consistently. In some cases it was apparently depressed; in others there was a slight increase.

2. The animals receiving meat grew more rapidly and at maturity weighed about 20 per cent more than their controls.

3. Recovery from starvation was more rapid and complete in meat-fed animals than in their controls.

I wish to acknowledge my indebtedness to Professors R. G. Hoskins and Clayton S. Smith for advice and suggestions and to Mr. W. M. Stevenson for assistance with the analytical work.

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# THE EFFECT OF MEAT FEEDING ON NURSING MOTHER RATS AND THE RATE OF GROWTH OF THEIR LITTERS[1]

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In a previous paper (Hitchcock, 1926) it has been reported that albino rats grow faster and weigh more at maturity when they are given liberal amounts of meat in addition to their regular ration, although the latter contains an adequate amount of protein. These results are not in accordance with those reported by Reader and Drummond (1925) who found that an increase in the percentage of casein contained in the food resulted in decreasing the rate of growth although no pathological conditions were noted even when a ration containing as high as 90 per cent protein was fed to the rats. The experiments from this laboratory thus far reported have all been carried out on mature and half-grown rats. In order to ascertain how early this increased growth resulting from meat feeding could be produced, experiments were made on the effect of feeding meat to the mothers of newborn litters of albino rats.

The experiments were started early in the spring of 1925, beginning with four full-grown females that had been obtained from the Wistar Institute. These were mated with males from the same stock. The first litter born was taken arbitrarily as a control litter, the second as a meat-fed litter, the third control and the fourth meat-fed. The mothers of the meat-fed litters were given meat for the first time on the days their litters were born. At first the quantity of meat given was from 8 to 10 grams per day. However, as the young became larger the amount was increased and when they were large enough to begin to eat, the quantity of meat given was sufficient so that each could have a gram or two daily. A daily record of the weights of both the mothers and their litters was kept. After weaning the meat-fed litters were kept on the meat diet, and when they reached maturity five of the females were selected for breeding. Their litters were again taken as meat-fed litters, the procedure being the same as that indicated for the first generation. In the same way the young rats in the control litters were raised and became the parents of the second generation of control rats. The experiment was carried on in this way

[1] The expenses incurred in carrying out this research were partly met by a grant from the Department of Nutrition of the Institute of American Meat Packers.

through three generations. In all there were eleven control and thirteen meat-fed litters.

In the third generation a slight change was made in the procedure. Here two meat-fed mothers were taken with their litters as a control group. Meat feeding was discontinued the day their litters were born. Conversely the mothers of two of the meat-fed litters were selected from among the descendants of the control rats. Meat feeding was begun here again on the

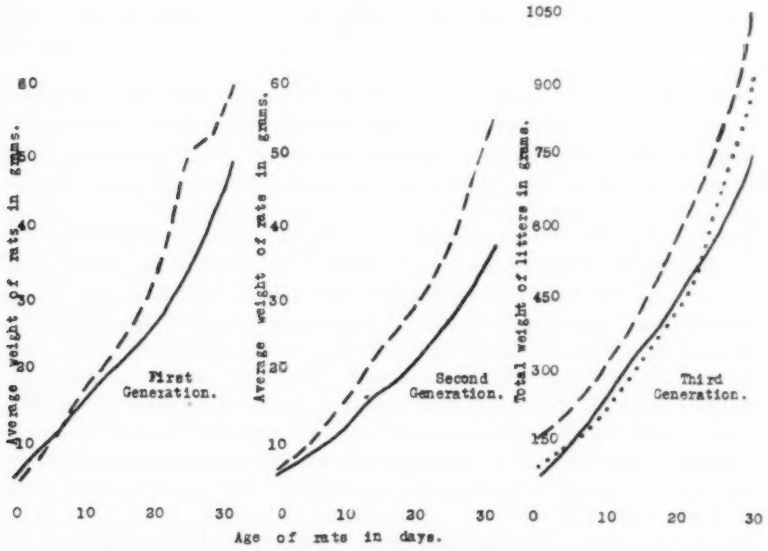
Average weight of rats in grams.
Average weight of rats in grams.
Total weight of litters in grams.
First Generation.
Second Generation.
Third Generation.
Age of rats in days.

Fig. 1. Graphs showing the effect of adding meat to the diet of mother rats on the growth of their young. Broken line shows the growth of the offspring of meat-fed mothers; continuous line represents the growth of the offspring of control mothers. The control mothers received an adequate diet but no meat. The dotted line used in the third generation shows the growth of the young of mothers that were controls previous to and meat-fed following the birth of their litters. The control mothers in this generation had been meat-fed up to the time of the birth of their litters.

day the litters were born. The growth curves of the young rats are plotted in figure 1. In order to simplify the data the weights are given only for every third day, although the animals were actually weighed daily.

The graphs show clearly that the litters of meat-fed mothers grew more rapidly in the first two generations. In the third generation the control litters have a slight advantage over the meat-fed animals in average weight. It must be remembered, however, that these mothers had been meat-fed all their lives up to the day that their litters were born and it seems quite likely that the effect of meat feeding persisted

for some time after its discontinuance. Furthermore the two control litters in the third generation were smaller than any of the litters in the meat-fed group and Donaldson (1925) has shown that rats in relatively small litters grow much faster than rats in larger litters. When the total weights of the litters are considered, the beneficial results of meat feeding are at once evident. The total weight of the two control litters at the end of thirty days was 739 grams, of the two meat-fed litters whose mothers had previously been control animals 898 grams, and of the two litters whose mothers received meat throughout their entire lives 1034 grams. Considering these facts the figures seem to justify the conclusion that the offspring of white rats grow faster when their mothers are fed meat.

The death rate among the control litters was much higher than that among the meat-fed litters. During the second generation there was a slight epidemic of respiratory infection in the colony. This resulted in the death of a total of twenty-seven of the young in control litters. During the same period only five of the meat-fed young succumbed. Furthermore one of the mothers in the control series died during this same period.

The average body weight of the meat-fed mothers was higher by about 6.5 per cent than that of the mothers of the control series during the second generation. This agrees with the results already reported on meat feeding of adult rats. In the third generation the average weight of meat-fed and control mothers was almost the same. However, as has already been mentioned, two of the meat-fed mothers in this generation were really control rats. Their weights were 170 and 200 grams, thirty days after the birth of their litters. The weights of the remaining meat-fed mothers were 240 and 259 grams, while the weights of the two control mothers which had been fed meat previous to the birth of their litters were 228 and 200 grams. It seems quite clear from these figures that the advantage in body weight is decidedly with the meat-fed rats.

## SUMMARY

It is shown in this paper that female rats that were nursing litters weighed more and raised larger and healthier litters when they were fed meat in addition to an adequate balanced ration. The rate of growth of the newborn rats was faster when their mothers received meat.

I wish to acknowledge my indebtedness to Prof. R. G. Hoskins for advice and suggestions in this investigation.

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# STUDIES OF BASAL METABOLISM IN NEW ORLEANS

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While running daily basal metabolism tests in checking up the influence of food intake, there were several days on which duplicate runs were made on the same subject. It was noticed that there was a consistent difference between the value obtained for the first run and that of the second. In each case, the value for the second run was lower than the first.

In the most reliable surveys on basal metabolism (3), (15), the rule given is to run the test at a period of from 14 to 17 hours after the last meal. According to the duplicate tests mentioned above, the value we received for the 17- or 18-hour run was lower than that of either the 14- or 15-hour run or the 19- to 20-hour run (table 1). According to these figures, how could one run be made, at say 17 hours after the last meal and that be called a basal? In order to study this shift in values more closely, the following procedure was carried out.

Experiments were made at approximately one and one-half hour intervals from 13.5 to 19 or 20 hours after the last meal. The method used was of the type described by Liljestrand (14). Outdoor air was breathed and the expired air was led to a wet gas meter through an air mixer from which a continuous sample was taken. Analyses of the expired air were made with a Haldane apparatus. In analyzing these samples, the temperatures of the room and of the water bath of the sampling burette were noted. Corrections for gas volumes were made in every case in which the temperature varied over 0.1 degree.[1]

The subjects, seven men, 1 to 7, and two women, 8 and 9, were normal, healthy individuals, engaged in student or teaching work. They came to the laboratory at about 7:45 a.m., rested on a cot for approximately 30

[1] With subject 4, the ventilation per minute was checked for 20 minutes after taking the mouthpiece. There was a slight variation for ventilation from 8 to 20 minutes time, but on analysis of sample taken from 8 to 13 and 14 to 19 minutes, there was an average variation of 3 cc. $O_2$ per minute. Duplicate analyses on same sample showed variation of $\pm$ 2 cc. $O_2$ per minute.

In several cases, readings were taken using the Benedict Portable or the Tissot gasometer following readings with above method. These values checked closely those already determined.

to 45 minutes, took the mouthpiece for 10 minutes and the first experiment was run at about 8:20. In most cases the subject remained on the cot during the entire period of the experiment. In some cases the subject attended classes between experiments, but then the interval between readings was longer.

The results are expressed in calories per square meter of body surface per hour. The body surface was calculated by the Du Bois formula (9).

The following tables and graphs give the results of three to six runs per day on individual subjects.

In every case except one run of subject 3 (run 2), there was a definite drop to a minimum and a rather sharp rise back to, and, in most cases, above the value of the first run. In some cases the per cent drop is small and would ordinarily be ignored, but in others, as subject 9 (run 1), it is too great to be neglected. This is especially true when there is a definite drop and then a rise.

TABLE 1

| SUBJECT | CALORIES PER HOUR | | |
|---|---|---|---|
| | 14.5 | Minimum | Maximum |
| 1 | 33.5 | 32.2 | 33.7 |
| 2 | 33.0 | 31.7 | 34.7 |
| 3 | | 34.0 | 40.8 |
| 4 | 36.0 | 32 0 | 36 0 |
| 5 | 36.1 | 33.3 | 35.5 |
| 6 | 33.4 | 33.4 | 37.5 |
| 7 | | 29.0 | 31.9 |
| 8 | 36.4 | 28.5 | 33.7 |
| 9 | 36.5 | 32.8 | 39.3 |

In checking the values given by Benedict et al. (2), we find that some show the same tendency but it is not at all regular while here it is a regular occurrence.

The time at which the minimum value occurs depends upon the kind of meal eaten the night before, both the kind and quantity of food having an effect. The discussion of this point will appear in a later paper. After a heavy meal, the minimum is reached later than after a light one. With subject 4 (runs 1, 3 and 4) a pint of milk and eight crackers were eaten at 6 p.m., the main meal being at noon. With runs 2 and 5, the heavy meal was eaten at 6 p.m., but in run 5 there were the two intervening days of runs 3 and 4 which caused the low values to appear at 14.3 hours (earlier than usual).

The minimum value represents the metabolism of the body when digestion is over and absorption is at its lowest ebb. The point at which this

TABLE 2

| SUBJECT | °C. | HOURS | PULSE | VENTILATION | PER CENT $CO_2$ | PER CENT $O_2$ | R.Q. | CUBIC CENTIMETERS PER MINUTE | | Cal. $M^2$/hr. |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | $CO_2$ | $O_2$ | |
| 1 | 4.44 | 14.6 | 47 | 5.91 | 2.77 | 3.17 | 0.874 | 164 | 187 | 33.51 |
| | | 16.0 | 45 | 6.25 | 2.65 | 2.94 | 0.902 | 166 | 184 | 33.22 |
| | | 17.3 | 46 | 6.12 | 2.45 | 3.03 | 0.810 | 150 | 185 | 32.65 |
| | | 18.3 | 48 | 6.18 | 2.56 | 2.90 | 0.883 | 158 | 179 | 32.16 |
| | | 19.4 | 46 | 6.49 | 2.62 | 2.87 | 0.913 | 172 | 186 | 33.70 |
| 2 | 3.33 | 15.8 | 60 | 4.78 | 4.04 | 4.53 | 0.892 | 193 | 217 | 31.74 |
| | | 18.1 | 60 | 4.33 | 3.92 | 5.40 | 0.726 | 170 | 234 | 32.80 |
| | 2.22 | 13.6 | 60 | 4.76 | 3.98 | 5.27 | 0.755 | 190 | 251 | 35.42 |
| | | 14.9 | 60 | 5.95 | 2.87 | 3.98 | 0.721 | 171 | 237 | 33.15 |
| | | 18.3 | 64 | 5.11 | 3.95 | 4.23 | 0.932 | 202 | 216 | 31.90 |
| | 7.78 | 14.2 | 60 | 4.75 | 4.18 | 4.73 | 0.884 | 199 | 224 | 32.70 |
| | | 15.6 | 60 | 4.42 | 4.41 | 5.11 | 0.863 | 195 | 225 | 32.65 |
| | | 17.0 | 64 | 5.04 | 3.99 | 4.77 | 0.836 | 201 | 241 | 34.75 |
| 3 | 10.0 | 14.0 | 84 | 4.60 | 3.62 | 4.33 | 0.836 | 167 | 199 | 35.68 |
| | | 16.8 | 76 | 4.51 | 3.54 | 4.41 | 0.803 | 160 | 199 | 35.38 |
| | | 19.3 | 76 | 4.76 | 3.20 | 4.16 | 0.770 | 152 | 198 | 34.88 |
| | | 21.5 | 76 | 4.80 | 3.28 | 4.49 | 0.731 | 157 | 216 | 37.68 |
| | | 23.5 | 84 | 4.95 | 3.48 | 4.72 | 0.737 | 172 | 234 | 40.79 |
| | 8.89 | 14.6 | 75 | 4.73 | 3.41 | 4.00 | 0.852 | 161 | 189 | 34.00 |
| | | 15.9 | 72 | 4.98 | 3.23 | 3.84 | 0.841 | 161 | 191 | 34.26 |
| | | 17.1 | 72 | 4.91 | 3.17 | 3.96 | 0.800 | 156 | 194 | 34.42 |
| | | 18.2 | 76 | 5.06 | 3.03 | 3.77 | 0.804 | 153 | 191 | 33.95 |
| 5 | 7.78 | 14.0 | 70 | 5.54 | 3.36 | 3.88 | 0.866 | 186 | 215 | 36.10 |
| | | 16.0 | 69 | 5.43 | 3.17 | 3.87 | 0.820 | 183 | 210 | 34.85 |
| | | 17.5 | 68 | 5.87 | 3.17 | 4.18 | 0.758 | 186 | 204 | 33.30 |
| | | 18.5 | 70 | 5.07 | 3.24 | 4.30 | 0.754 | 164 | 218 | 35.53 |
| 6 | 15.56 | 14.0 | 66 | 5.94 | 3.03 | 3.51 | 0.864 | 180 | 208 | 33.35 |
| | | 15.3 | 66 | 6.66 | 3.13 | 2.89 | 1.084 | 208 | 192 | 31.83 |
| | | 16.5 | 70 | 6.18 | 3.08 | 3.33 | 0.931 | 190 | 205 | 33.41 |
| | | 17.5 | 66 | 6.27 | 3.04 | 3.37 | 0.902 | 191 | 211 | 34.15 |
| | | 18.8 | 70 | 5.90 | 3.22 | 4.04 | 0.797 | 190 | 238 | 37.51 |
| 7 | 8.89 | 16.3 | 56 | 5.28 | 2.66 | 2.86 | 0.930 | 140 | 151 | 26.60 |
| | | 18.5 | 56 | 5.48 | 2.55 | 3.05 | 0.836 | 140 | 167 | 28.75 |
| | 11.11 | 17.3 | 54 | 5.47 | 2.94 | 2.96 | 0.993 | 161 | 162 | 29.00 |
| | | 18.6 | 54 | 5.33 | 2.54 | 3.28 | 0.774 | 135 | 175 | 29.65 |
| | | 20.1 | 54 | 5.12 | 2.57 | 3.61 | 0.712 | 132 | 185 | 30.82 |
| | | 21.5 | 52 | 5.42 | 2.88 | 3.40 | 0.847 | 156 | 185 | 31.95 |
| 8 | 8.89 | 14.1 | 72 | 4.40 | 3.61 | 4.78 | 0.755 | 159 | 210 | 36.38 |
| | | 15.6 | 68 | 3.94 | 3.26 | 4.14 | 0.788 | 129 | 163 | 28.50 |
| | | 17.3 | 71 | 4.40 | 3.57 | 4.34 | 0.823 | 157 | 191 | 33.66 |

TABLE 2—*Continued*

| SUBJECT | °C. | HOURS | PULSE | VENTILATION | PER CENT $CO_2$ | PER CENT $O_2$ | R.Q. | CUBIC CENTIMETERS PER MINUTE | | Cal. $M^2$/hr. |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | $CO_2$ | $O_2$ | |
| 9 | 17.78 | 13.5 | 76 | 4.35 | 2.83 | 4.46 | 0.642 | 123 | 194 | 37.00 |
| | | 15.2 | 76 | 4.71 | 3.17 | 3.74 | 0.848 | 149 | 176 | 34.85 |
| | | 16.9 | 76 | 4.09 | 3.05 | 4.17 | 0.731 | 125 | 171 | 32 85 |
| | | 18.7 | 74 | 4.31 | 2.88 | 3.97 | 0.726 | 124 | 171 | 32.80 |
| | | 20.4 | 80 | 4.96 | 3.01 | 3.97 | 0.758 | 149 | 198 | 38.30 |
| | | 21.8 | 82 | 4.65 | 2.82 | 4.42 | 0.638 | 131 | 206 | 39.30 |
| | 8.90 | 14.6 | 72 | 4.23 | 3.37 | 4.47 | 0.754 | 143 | 189 | 36.50 |
| | | 15.8 | 72 | 4.33 | 3.21 | 4.04 | 0.795 | 139 | 175 | 34.20 |
| | | 17.1 | 70 | 4.32 | 3.10 | 3.91 | 0.793 | 134 | 169 | 33.00 |
| | | 18.7 | 74 | 4.37 | 3.20 | 3.79 | 0.845 | 140 | 167 | 33.10 |
| 4 | 12.22 | 19.3 | 80 | 4.96 | 3.22 | 4.18 | 0.770 | 160 | 207 | 32.45 |
| | | 22.5 | 81 | 5.59 | 3.19 | 4.43 | 0.720 | 178 | 248 | 38.40 |
| | 13.89 | 14.8 | 78 | 5.12 | 3.13 | 4.27 | 0.733 | 160 | 218 | 33.85 |
| | | 16.3 | 78 | 5.28 | 3.02 | 4.06 | 0.744 | 159 | 215 | 33.45 |
| | | 17.5 | 83 | 5.63 | 3.02 | 3.55 | 0.856 | 170 | 200 | 32.00 |
| | 14.44 | 20.7 | 79 | 5.58 | 2.89 | 3.69 | 0.778 | 161 | 206 | 32.35 |
| | | 24.3 | 82 | 5.52 | 3.17 | 4.29 | 0.739 | 175 | 237 | 36.85 |
| | 15.56 | 19.5 | 80 | 5.04 | 3.10 | 4.06 | 0.764 | 156 | 205 | 32.10 |
| | | 21.2 | 79 | 5.38 | 2.98 | 4.29 | 0.695 | 160 | 231 | 34.62 |
| | | 22.6 | 78 | 5.50 | 3.11 | 4.31 | 0.722 | 171 | 237 | 36.70 |
| | 13.33 | 14.3 | 76 | 5.18 | 3.11 | 3.65 | 0.852 | 161 | 189 | 30.30 |
| | | 17.7 | 74 | 5.51 | 2.69 | 3.74 | 0.719 | 148 | 206 | 31 90 |
| | | 20.5 | 78 | 5.68 | 2.92 | 4.16 | 0.702 | 166 | 236 | 36.36 |
| | 15.56 | 13.5 | 82 | 5.57 | 3.44 | 3.97 | 0.867 | 192 | 221 | 35.51 |
| | | 15.5 | 82 | 5.52 | 3.22 | 3.83 | 0.841 | 178 | 211 | 33.68 |
| | | 17.8 | 82 | 5.47 | 3.21 | 3.76 | 0.854 | 176 | 206 | 33.00 |
| | | 19.7 | 79 | 5.82 | 3.19 | 3.77 | 0.846 | 186 | 219 | 35.01 |
| | | 21.6 | 76 | 6.58 | 3.20 | 3.65 | 0.877 | 211 | 240 | 38.59 |
| | | 23.6 | 86 | 5.82 | 3.17 | 4.12 | 0.770 | 185 | 240 | 37.64 |
| | 9.48 | 13.5 | 82 | 5.13 | 3.09 | 4.52 | 0.684 | 159 | 231 | 34.62 |
| | | 15.5 | 79 | 5.35 | 3.02 | 3.85 | 0.785 | 162 | 206 | 32.40 |
| | | 17.3 | 76 | 5.53 | 3.27 | 3.58 | 0.913 | 181 | 198 | 32.18 |
| | | 19.0 | 78 | 5.49 | 2.98 | 3.75 | 0.795 | 164 | 206 | 32.50 |
| | 9.48 | 13.1 | 80 | 5.34 | 3.16 | 4.31 | 0.733 | 169 | 230 | 35.69 |
| | | 14.3 | 76 | 5.60 | 3.19 | 4.09 | 0.780 | 179 | 229 | 36.00 |
| | | 15.5 | 78 | 5.22 | 3.12 | 3.85 | 0.811 | 163 | 201 | 31.82 |
| | | 18.3 | 76 | 5.55 | 3.26 | 3.88 | 0.841 | 181 | 215 | 34.32 |
| | 5.56 | 14.3 | 78 | 5.79 | 3.16 | 4.07 | 0.777 | 183 | 236 | 37.08 |
| | | 15.6 | 80 | 5.92 | 3.08 | 3 27 | 0 942 | 182 | 193 | 31 60 |
| | | 17.2 | 80 | 5.40 | 3.12 | 4.08 | 0.765 | 168 | 220 | 34.45 |
| | | 18.6 | 78 | 5.81 | 3.07 | 4.11 | 0.747 | 178 | 231 | 36.00 |
| | 6.67 | 13.8 | 78 | 6.13 | 3.18 | 3.76 | 0.846 | 195 | 230 | 36.80 |
| | | 15.6 | 76 | 5.58 | 3.15 | 3.77 | 0.835 | 176 | 211 | 33.61 |
| | | 17.5 | 76 | 5.28 | 3.27 | 4.18 | 0.782 | 173 | 221 | 34.75 |

TABLE 2—*Concluded*

| SUBJECT | °C. | HOURS | PULSE | VENTILATION | PER CENT $CO_2$ | PER CENT $O_2$ | R.Q. | CUBIC CENTIMETERS PER MINUTE | | Cal. $M^2$/hr. |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | $CO_2$ | $O_2$ | |
| | 15.56 | 14.5 | 78 | 6.02 | 3.29 | 3.66 | 0.899 | 198 | 220 | 35.60 |
| | | 16.0 | 74 | 5.31 | 3.30 | 3.98 | 0.829 | 175 | 211 | 33.60 |
| | | 17.3 | 72 | 5.34 | 3.21 | 3.87 | 0.830 | 170 | 206 | 32.80 |
| | | 18.5 | | 5.16 | 3.34 | 3.88 | 0.861 | 172 | 200 | 32.10 |
| | | 19.9 | 78 | 5.60 | 3.14 | 3.93 | 0.799 | 176 | 220 | 34.80 |

absorption is at its lowest ebb is probably influenced by the muscular activity and the general "fitness" of the subject. Harris and Benedict (13) state that metabolism is variable from day to day and this drop may account for part of this daily variation—the observation being made at a time when absorption may or may not have been complete. The observa-

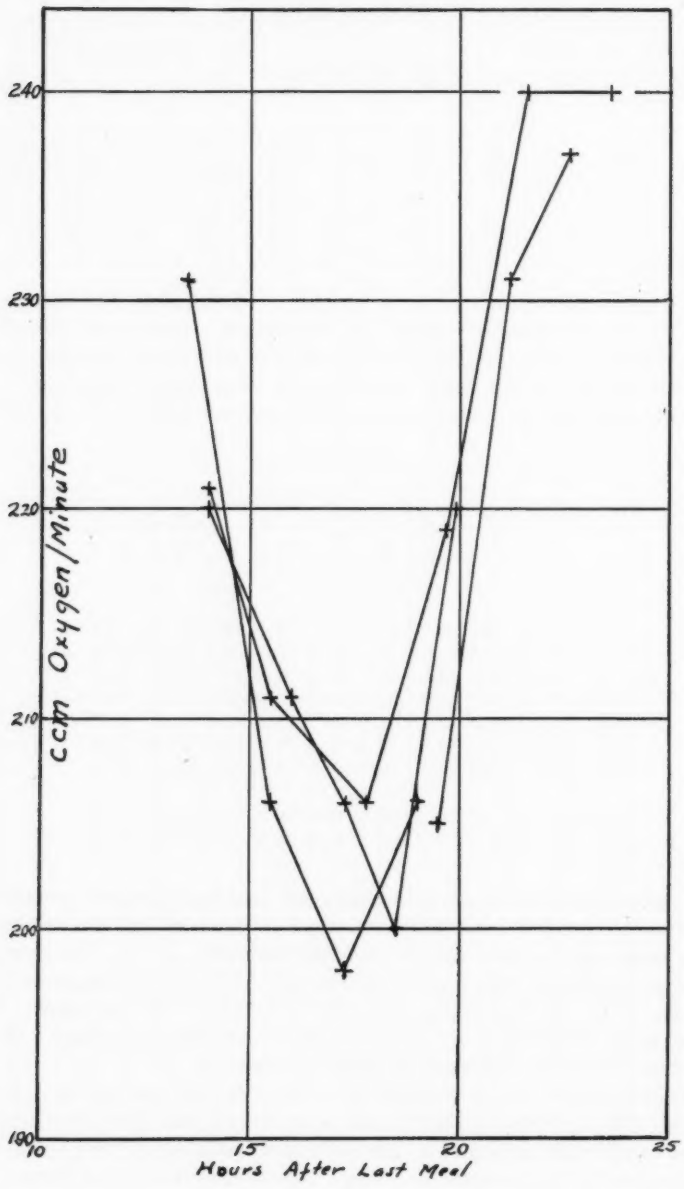
TABLE 3

| SUBJECT | AGE | HEIGHT | WEIGHT | S.A. $M^2$ | FOUND $M^2$/hr. | DuB. | HEAT PRODUCTION PER HOUR | | | | PER CENT VARIATION (−) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Found | DuB. | H.B. | Drey. | DuB. | H.B. | Drey. |
| | *years* | *cm.* | *kgm.* | | | | | | | | | | |
| 1 | 24 | 165.1 | 59.0 | 1.637 | 32.2 | 39.5 | 52.7 | 64.7 | 64.1 | 65.3 | −18.6 | −17.8 | −19.3 |
| 2 | 25 | 182.9 | 80.3 | 2.016 | 31.7 | 39.5 | 63.9 | 79.7 | 79.8 | 75.8 | −19.8 | −19.9 | −15.6 |
| 3 | 25 | 170.2 | 54.4 | 1.622 | 34.0 | 39.5 | 55.2 | 64.1 | 62.4 | 62.4 | −13.9 | −11.5 | −11.4 |
| 4 | 35 | 170.2 | 71.2 | 1.823 | 32.0 | 39.5 | 58.4 | 72.1 | 69.1 | 68.2 | −19.0 | −15.5 | −14.3 |
| 5 | 19 | 182.9 | 56.7 | 1.750 | 33.3 | 41.0 | 58.1 | 71.6 | 68.1 | 66.1 | −18.9 | −14.7 | −12.0 |
| 6 | 24 | 177.8 | 66.7 | 1.826 | 33.4 | 39.5 | 61.0 | 72.1 | 71.6 | 69.4 | −15.4 | −14.8 | −12.1 |
| 7 | 35 | 172.0 | 56.7 | 1.689 | 29.0 | 39.5 | 49.0 | 66.7 | 61.3 | 60.8 | −26.4 | −20.1 | −19.5 |
| 8 | 30 | 168.0 | 57.1 | 1 644 | 28.5 | 36.5 | 46.8 | 60.0 | 56.8 | 55.7 | −22.0 | −17.6 | −15.9 |
| 9 | 29 | 163.0 | 46.3 | 1.473 | 32.8 | 37.0 | 48.3 | 54.5 | 52 7 | 50.8 | −11.3 | − 8.3 | − 4.9 |

tions given here were made when the conditions were as near identical as possible. Later work will show the effect of increased protein intake.

In checking up these results with the standards in use, the average minimum value for each individual was used. These were compared with the Du Bois (9), Harris-Benedict (12) and Dreyer (8) formulae. The basal metabolism for every subject without exception was found to be less than any other of the standards given (table 3).

Whether or not the basal metabolism is lower in places having a warm climate than in those of cooler climate seems to be a point yet to be decided. Most of the work in regard to this difference has been in the tropics, the subjects being both natives and white men who were residing there.


240
230
220
210
200
190
10
15
20
25
ccm Oxygen/Minute
Hours After Last Meal

Caspari et al. (4), (5) report that the metabolism of several men living in Berlin did not change when they had lived for a time in Africa. Eijkman (10), (11) also claims that the basal metabolism of inhabitants of places having warm climates is not markedly different from that of temperate regions.

On the other hand, however, Corlette's figures (6) show the metabolism of people in Australia lower than in New York; de Almeida (1) reports values of metabolism from the tropics which are as much as 20 per cent lower than the standards accepted. Also, McConnell et al. (16) report a minimum metabolism occurring at temperatures of about 75 to 83°F.

According to table 3, this paper, the metabolism of individuals living in New Orleans is definitely lower than the values given by three sets of standards, Du Bois, Harris-Benedict and Dreyer, the average deviation from the standard being −18 per cent, −16 and −14 per cent, respectively.

The values of McConnell are higher for temperatures of 75 to 83°F. than those of New Orleans of about 70°F. but it would be expected for the subjects here were subjected to a "warm chamber" while in subtropical and tropical climates, the whole metabolism has been brought to equilibrium at this higher temperature. The basal metabolism, as stated by de Almeida, is affected by all factors which modify the daily metabolism, such as food intake, muscular activity and temperature.

It has been shown that the protein intake in New Orleans is approximately 60 per cent of that of the northern standards, and whether this is one of the factors for a lowered metabolism remains to be studied. The whole subject of metabolism in tropical and semitropical climates is one that is much in need of investigation and the validity of applying standards which have been determined in temperate climates is open to question.

## SUMMARY

1. This series includes 88 experiments on nine subjects.

2. The metabolism varies with time after the last meal.

3. The minimum value remains approximately stable from day to day, but the time at which it falls varies according to the kind and quantity of the food eaten.

4. In comparing average minimum values with the standards in use, Du Bois, Harris-Benedict and Dreyer, the metabolism of individuals living in New Orleans is definitely lower, the average deviation from these standards being −18 per cent, −16 per cent and −14 per cent, respectively.

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# STUDY OF THE PROTEIN INTAKE AS INDICATED BY URINARY NITROGEN

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In the last few years, (4), (5), (8), there appears to be reported a gradual shift in the normal protein intake, especially with those doing sedentary work. This level seems to be somewhere between that of Chittenden (2) and Atwater (1).

It is a question whether it is caused by a lowered food intake or a shift in the dietary. The question of a general lowered food intake and resulting lowered metabolism has been discussed by Greenwald (6). In reference to the climatic effects and lowered food intake, he states (p. 389), "It is quite possible that the inability to maintain a high metabolism in warm weather and in the tropics is responsible for the indolence and lack of energy displayed by man under those conditions." What the effects of increased temperature, as in the summer, on the protein intake and how much it can be increased without physical discomfort, will be discussed in this and the two following papers. In 1924 Denis and Borgstrom (4) made a study on the effects of temperature on protein intake, using the data supplied by 24-hour urine collections of 233 male medical students. These students were either eating at home, boarding house, fraternity house, or dining hall of the University. The data used in calculating these results will be used in connection with this work, especially considering the effects of temperature and place of eating.[1]

In this work, the urinary nitrogen was determined on daily 24-hour urines of the subjects from March 17, 1925, to March 19, 1926. The collections were made from 7 a.m. to 7 a.m. Analyses were made by the Kjeldahl method.

[1] Grams urinary nitrogen calculated for 70 kilo body weight of 233 male medical students, arranged according to boarding place.

| | TEMPERATURE | | | |
|---|---|---|---|---|
| | 13.05 °C. | 21.00 °C. | 22.94 °C. | 26.77 °C. |
| Home | 12.38 | 12.47 | 11.46 | 8.61 |
| Boarding house | 11.84 | 10.76 | 10.74 | 11.55 |
| University dining hall | 10.14 | 9.70 | 9.58 | 8.44 |

The temperature values are those given by the local United States Weather Bureau.

The subjects were numbers 3 and 4 of the previous paper by Hafkesbring and Borgstrom (7). Subject 3 had been in New Orleans from September, 1924; previous to that he had lived in North Carolina. Subject 4 had lived in New Orleans since the fall of 1921, except for a month during August, 1924; previous to that he had lived on the Pacific Coast.

During the period of the experiment, from March 17 to June 2, subject 3 ate three meals a day at a boarding house in New Orleans; during June and July, three meals a day at a boarding house in Chapel Hill, N. C., and was at home in Rockwell, N. C. during August. From September, 1925, to March 19, 1926, he ate two meals a day at a boarding house (not the same as in the spring) and one meal at the University dining hall. Subject 4 ate at home from March, 1925, to October 1, and at the University dining hall from October 1 to March 19, 1926. Subject 3 did not change in weight (120 pounds) from the beginning to the end of the period.

TABLE 1

*Average urinary nitrogen for subject 3 calculated for 70 kilo body weight*

| DATE | °C. | $N_2$ | DATE | °C. | $N_2$ |
|---|---|---|---|---|---|
| *1925* | | *grams* | *1925* | | *grams* |
| March 17–31 | 18.39 | 8.80 | September 20–30 | 27.90 | 9.56 |
| April 1–30 | 22.44 | 9.70 | October 1–13 | 25.80 | 10.54 |
| May 1–14 | 22.50 | 10.03 | November 1–15 | 17.61 | 9.11 |
| May 15–31 | 25.06 | 12.63 | December 1–15 | 15.39 | 9.66 |
| June 15–30 | 22.39 | 13.33 | *1926* | | |
| July 1–25 | 27.22 | 13.59 | January 1–10 | 10.67 | 9.29 |
| August 1–31 | 25.50 | 8.98 | February 1–14 | 15.11 | 10.30 |
| | | | March 1–17 | 12.89 | 9.68 |

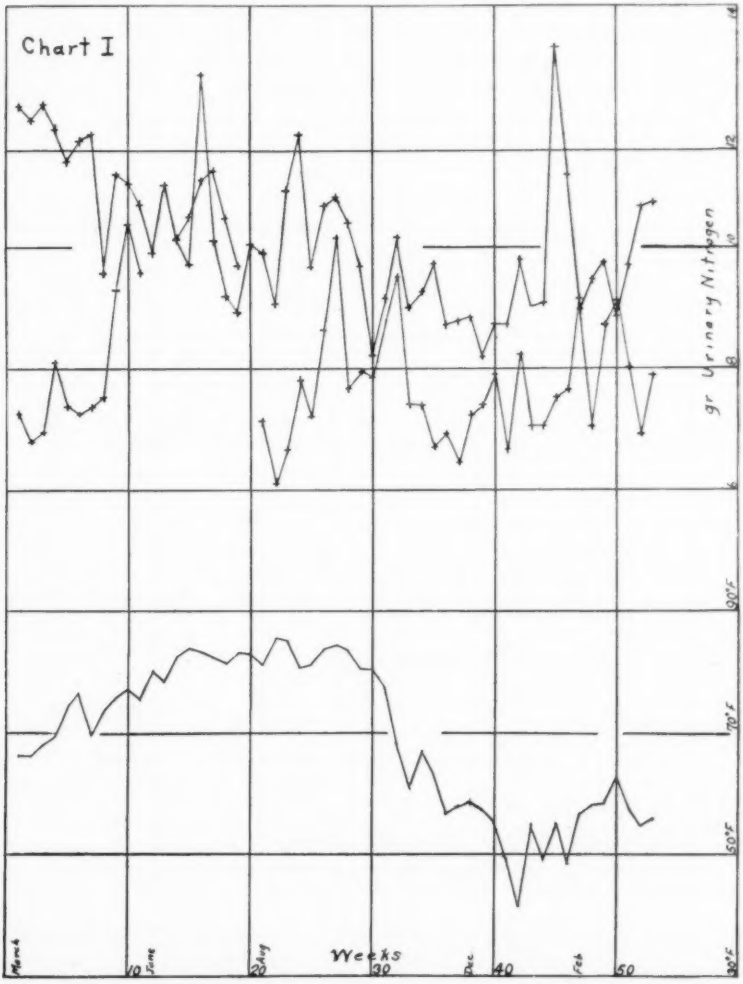
TABLE 2

*Average urinary nitrogen for subject 4*

| DATE | °C. | $N_2$ 70 KILO | DATE | °C. | $N_2$ 70 KILO |
|---|---|---|---|---|---|
| | | *grams* | | | *grams* |
| March 18–31 | 19.5 | 11.44 | October 17–31 | 18.50 | 9.05 |
| April 16–30 | 23.8 | 10.81 | November 16–30 | 13.89 | 8.33 |
| May 17–31 | 24.7 | 10.14 | December 17–31 | 6.95 | 8.61 |
| June 17–31 | 28.5 | 9.62 | January 1–15 | 10.95 | 8.54 |
| July 17–31 | 28.4 | 8.44 | February 1–14 | 14.55 | 8.98 |
| August 1–15 | 28.3 | 9.01 | March 1–15 | 12.95 | 9.68 |
| September 16–30 | 28.4 | 9.58 | | | |

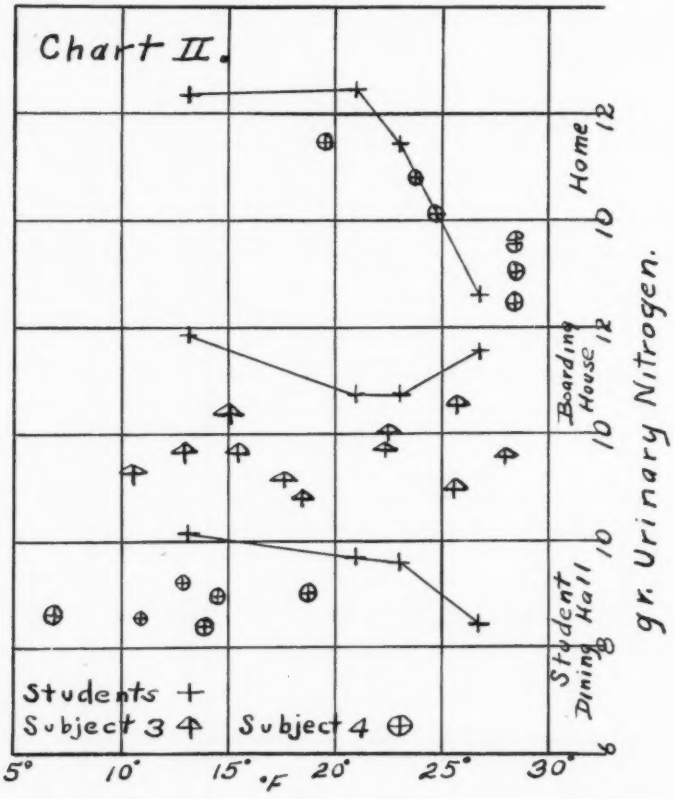
Average March to September ..... 9.88 grams
October to March ..... 9.10 grams

Subject 4 weighed 165 pounds from March 17 to September 15. During the latter part of September, subject 4 lost 5 pounds in weight doing hard physical work in exceedingly warm weather. This weight (160 pounds)




remained constant to the end of the observations. There was no question of an economic nature involved in this work as the subjects ate as much or as little as they wanted and had the ability to purchase any additional food desired.

Tables 1 and 2 and chart 1 show the results obtained for 15 day averages. On chart 1 there are given values (see footnote 1) from the work of Denis and Borgstrom (4) to be used in comparison. These results show that when eating at home, a person has greater leeway than when eating at boarding houses; that is, he obtains the food he desires. When eating at boarding places, one eats what is set before him and usually that remains fairly constant. With subject 4, when eating at home, the average urinary




nitrogen was 9.88 grams for 70 kilos. When eating at the University dining hall, in winter weather, it was 9.10 grams for 70 kilos. The effect of temperature is well shown by subject 4 when at home, but there seems to be little variation when eating at the University dining hall in winter weather. This may not be due to the food but to the fact that below 70°F., or 21°C., the effect of temperature does not enter to any appreciable extent.

Table 3 and chart 2 show the average daily urinary nitrogen for each week beginning with Monday. The values for the 13th and 16th week in chart 2 show where subject 4 did manual labor in excess of his usual occupation. In these cases, the excess urinary nitrogen was 1 and 3 grams above that expected from the curve. Subject 4 had an attack of grippe during the 8th week with a resulting drop. Weeks 24, 27 and 45 were weeks during which basal metabolism and food experiments were done. A

TABLE 3

| SUBJECT 4 | | SUBJECT 3 | | | SUBJECT 4 | SUBJECT 3 |
|---|---|---|---|---|---|---|
| °F. temperature | $N_2$ | °F. temperature | $N_2$ | °F. temperature | $N_2$ | $N_2$ |
| 66.2 | 12.34 | 66.2 | 7.26 | 83.6 | 10.40 | 7.66 |
| 66.3 | 12.10 | 66.3 | 6.78 | 80.6 | 9.69 | 7.96 |
| 68.2 | 12.47 | 68.2 | 6.96 | 80.4 | 8 22 | 7.87 |
| 69.2 | 11.95 | 69.2 | 8.14 | 77.4 | 9.17 | 8.80 |
| 74.2 | 11.39 | 74.2 | 7.37 | 68.3 | 10 18 | 9.51 |
| 76.7 | 11.76 | 76.7 | 7.26 | 60.7 | 9 00 | 7.21 |
| 69.7 | 11.86 | 69.7 | 7.35 | 67.6 | 9.27 | 7.19 |
| 73.6 | 9.55 | 73.6 | 7.55 | 63.3 | 9.76 | 6.71 |
| 75.7 | 11.21 | 75.7 | 9.30 | 56.9 | 8.71 | 6.91 |
| 76 9 | 11.05 | 76.9 | 10.39 | 57.9 | 8.78 | 6.44 |
| 75.4 | 10.70 | 75.4 | 9.57 | 58.7 | 8.84 | 7.23 |
| 80.0 | 9.87 | | | 57.6 | 8.17 | 7.41 |
| 78.2 | 11.07 | | | 55.6 | 8.72 | 7.92 |
| 82.6 | 10.16 | 81.6 | 10.12 | 48.9 | 8.71 | 6.60 |
| 83.7 | 9.74 | 79.2 | 10.50 | 40.9 | 9.84 | 8.23 |
| 83.3 | 12.85 | 81 1 | 11 03 | 54.9 | 9.02 | 7.05 |
| 82.3 | 10 10 | 84.1 | 11 27 | 49.3 | 9.08 | 7.04 |
| 81.6 | 9.19 | 79.1 | 10.47 | 55.0 | 13.32 | 7.52 |
| 83.3 | 8.92 | 79.9 | 9.69 | 48.3 | 11.20 | 7 65 |
| 82.9 | 10.05 | | | 56.6 | 8.97 | 9.15 |
| 81.2 | 9.93 | 77.9 | 7.14 | 58.0 | 9.27 | 7.04 |
| 85.6 | 9.07 | 82.6 | 6.14 | 58.4 | 9.75 | 8.72 |
| 85.3 | 10.96 | 71.9 | 6 66 | 63.0 | 8.75 | 9.18 |
| 80 7 | 11.88 | 73.1 | 7.81 | 57.7 | 9.70 | 8.01 |
| 81.2 | 9.67 | | 7.21 | 54.7 | 10.67 | 6.92 |
| 83.9 | 10.70 | 83.9 | 8.62 | 55.7 | 10.75 | 7.89 |
| 84.3 | 10.85 | 84.3 | 10.17 | | | |

discussion of these will follow in later papers. In week 45, the subject ate all the protein he possibly could—changing from carbohydrate to protein as much as possible, but not excluding vegetables from the diet. The maximum reached for any day of the year was slightly over 16 grams. This could not possibly be maintained for any length of time, even for a few days. Subject 3 began a diet consisting of one quart of milk in addition to his regular diet during the 9th week, continuing through the 11th week.

He then continued this same diet at a boarding house at Chapel Hill, N. C., for weeks 14 to 19, inclusive. This higher protein diet averaged 13.2 grams nitrogen for 70 kilos body weight against 10.45 grams for the year. During weeks 27 and 32, basal metabolism experiments were done, a discussion of which will follow in later papers.

Table 4 shows the distribution for subjects 3 and 4 of the urinary nitrogen as actually found. This shows that subject 3, 50 per cent of the time, excreted 6.0 to 7.99 grams of nitrogen per day.

This table also shows that subject 4, when eating at home, excreted between 9.0 and 11.99 grams nitrogen, 68.8 per cent of the time; while at the University dining hall, the excretion was between 8.0 and 10.99 grams nitrogen, 71.2 per cent of the time.

TABLE 4

| URINARY $N_2$ | SUBJECT 3 | SUBJECT 4 | |
|---|---|---|---|
| | Boarding house | Home | University dining hall |
| *grams* | | | |
| 4–5 | 7 | | |
| 5–6 | 23 | | |
| 6–7 | 45 | | 7 |
| 7–8 | 67 | 4 | 21 |
| 8–9 | 35 | 19 | 38 |
| 9–10 | 32 | 34 | 53 |
| 10–11 | 4 | 60 | 30 |
| 11–12 | 7 | 38 | 11 |
| 12–13 | 2 | 25 | 5 |
| 13–14 | | 9 | 1 |
| 14–15 | | 3 | 2 |
| 15–16 | 1 | | 1 |
| 16–17 | | | 1 |

The data from daily urinary nitrogen values were tabulated as to the days of the week when the collection of urine stopped. That is, Monday values are the collection from Sunday, 7 a.m., to Monday, 7 a.m., and are influenced largely by food intake of Sunday if digestion is considered to be almost complete in the first ten hours and the subject in nitrogen equilibrium. Beginning on Monday, the values for subject 3 are 8.21, 8.30, 8.07, 7.84, 8.10, 8.18, 8.41 grams of urinary nitrogen per 24 hours with an average value for 311 collections of 8.15 grams. Beginning on Monday with subject 4, the values are 10.53, 10.14, 10.10, 10.13, 10.39, 10.01, 9.94, with an average of 10.15 grams for 362 collections. These values show that subject 3 has a decided peak for Sunday and a low value for Thursday. Subject 4 shows the highest value on Monday with another rise on Friday with the lowest value on Sunday. This difference from high to low for

both subject 3 and subject 4 is approximately 0.6 gram of urinary nitrogen or 7½ per cent variation and 6 per cent respectively for subjects 3 and 4. The cause of this fluctuation for subject 3 was probably due to the fact that his heavy school work was over Thursday and he was free to take exercise Friday and Saturday. Subject 4 usually spent Thursday or Sunday out in the open, often doing hard manual labor.

The medical students quoted here showed on high protein diet an average increase of urinary nitrogen of about 4.5 grams per 70 kilo body weight, or an increase of about 30 grams protein per day. This increase could be accomplished in the winter without intestinal upsets, but in the summer, the results were generally not so good. In one summer (1924) the increase was 3.5 grams for 70 kilos (12 subjects) with no attendant discomfort.

Whether or not this lower protein intake is just a peculiarity in New Orleans is a question. Doctor Hendrix (Texas Medical School, Galveston) kindly gave us the data from 29 collections of medical students at Galveston collected between January 15 and 25. These results showed an average 24-hour urinary nitrogen excretion of 12.02 grams for average body weight of 69.54 kilos. On 70 kilo basis this is 12.1 grams, or slightly over that from in New Orleans, but even this is lower than Northern standards.

The data available on food consumption per capita are rather difficult to obtain. This past winter a survey was made under the supervision of Mr. B. H. Crittchfield (3) of the agricultural situation of the New Orleans Trade Area. On page 20 he states, "The per capita consumption (of whole milk) probably averages between two-fifths to one-half pint per day. . . . . The average per capita consumption of milk and cream in all cities of the United States in 1924 was about one and one-fourth pints. The consumption in New Orleans is still much lower than other cities." Those knowing the South will feel that the statistics quoted do not show the entire picture. The values given do not state if it includes the condensed or evaporated milk used, and there are large amounts of these used in the South, due to climatic conditions. Also New Orleans has about 30 per cent colored and these colored consume very little fresh milk. Even correcting for the colored element, the per capita consumption of milk is lower than the average for the United States.

The lowered protein intake in the South may be due to natural adjustments of the diet to satisfy the conditions of temperature, humidity, etc. It may be due to a lowered dietary in general or adjustment toward proteins. This question will be discussed more in the following paper.

## SUMMARY

1. Twenty-four-hour urines were collected by two subjects for one year. These gave an average of 10 grams nitrogen for 70 kilo body weight.

2. The results show seasonal variation when eating at home, but questionable when eating at boarding houses.

3. Some daily variation is shown, probably due to working conditions.

4. Climatic conditions are the probable cause for the lowered protein intake.

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# DIETARY STUDIES IN NEW ORLEANS IN 1925

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Greenwald states "It is a generally accepted belief that less food is required in summer than in winter, and less in the tropics than in the temperate climates." If it be true that there is a lessened food intake in the tropics or warm climates, then the low values of Hafkesbring and Borgstrom (4) on basal metabolism should be substantiated by a study of food eaten, that is, the normal diet should be less. In this paper, results will be given of experiments that were done to show the number of calories eaten by a normal individual.

The subjects were numbers 3, 4 and 5, given by Hafkesbring and Borgstrom (4) and Borgstrom and Bost (2) in previous papers. Subject 5 had been living in New Orleans for the past four years. He was gradually increasing in weight, but during the experiments gained very slightly.

The diet chosen was one that all subjects could eat for several days and not dislike. It consisted of apples (Winesap), bananas, bread (Bond-General Baking Co.), butter, cheese (Kraft-American), eggs, milk, oatmeal (Quaker Oats Co.), potatoes, sugar and ripe tomatoes. The amount of food was weighed for each meal. The calorific value and nitrogen value were calculated using the values given by G. Lusk (6), except for the values given by Atwater and Bryant (1).

The first experiment was done August 24 to 28, 1925, inclusive, with subjects 4 and 5. From September 14 to 18, these experiments were repeated using subjects 3, 4 and 5. The values of fat, carbohydrate and protein eaten as well as calorific intake are given in table 1.

In the August experiments, subject 4 had a feeling Wednesday evening of eating more than he desired and Thursday decreased the food intake. Friday, the food intake was further lowered and the subject had no ill effects. Subject 5 at the same time showed no tendency to ill effects.

In the September experiments, subject 3 felt as though he had eaten too much on the first day and on reducing the diet had no effect. With subject 4 the food intake was too large the first day and on reduction to a value of about 2500 calories per day, the subject showed no ill effects. Subject 5 averaged 3280 calories per day with no ill effects though he stated that for several days afterwards he cared only for acid fruits and vegetables.

For subject 3, the fats supplied 40.8 per cent of the calories while protein supplied 12.4 per cent (in September). For subject 4, the fat percentage was 31 per cent and 28.1 per cent and protein 13.0 and 11.6 per cent for August and September respectively. For subject 5, the fat percentage was 34.7 and 32.6, with protein values of 13.8 and 12.0 for August and September, respectively. It is very interesting to note that the fat supplied one-third of the calories with fairly large variation, and the protein one-eighth of the calories with very small variations.

Table 2 gives the daily urinary nitrogen of subjects 3, 4 and 5 for the period August 22 to September 23. The values given are the collections

TABLE 1

| SUBJECT | DAY | AUGUST | | | | SEPTEMBER | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Fat | Carbo-hydrate | Protein | Calories | Fat | Carbo-hydrate | Protein | Calories |
| 4 | Monday | 91.3 | 368.0 | 89.4 | 2725 | 88.9 | 464.3 | 83.4 | 3070 |
| | Tuesday | 91.6 | 421.0 | 95.0 | 2965 | 92.9 | 383.6 | 86.8 | 2795 |
| | Wednesday | 107.7 | 418.4 | 99.0 | 3120 | 79.8 | 390.2 | 73.8 | 2645 |
| | Thursday | 98.3 | 367.7 | 91.4 | 2795 | 54.0 | 323.5 | 57.2 | 2060 |
| | Friday | 84.7 | 359.8 | 74.7 | 2570 | 80.1 | 365.2 | 71.5 | 2535 |
| | Average | 94.7 | 387.0 | 89.9 | 2835 | 79.1 | 385.4 | 74.5 | 2620 |
| 5 | Monday | 117.5 | 357.1 | 100.2 | 2970 | 113.9 | 487.8 | 104.1 | 3485 |
| | Tuesday | 115.4 | 392.5 | 106.4 | 3115 | 109.9 | 458.7 | 110.4 | 3354 |
| | Wednesday | 112.0 | 377.4 | 99.9 | 3000 | 116.9 | 400.5 | 89.1 | 3095 |
| | Thursday | 100.5 | 365.9 | 100.9 | 2840 | 118.2 | 403.9 | 84.6 | 3110 |
| | Friday | 120.8 | 407.2 | 104.0 | 3220 | 116.6 | 464.2 | 90.8 | 3360 |
| | Average | 113.2 | 380.0 | 102.3 | 3030 | 115.1 | 443.0 | 95.8 | 3280 |
| 3 | Monday | | | | | 129.6 | 311.8 | 89.7 | 2850 |
| | Tuesday | | | | | 117.7 | 293.0 | 91.6 | 2670 |
| | Wednesday | | | | | 122.7 | 297.9 | 85.0 | 2710 |
| | Thursday | | | | | 112.7 | 294.8 | 71.8 | 2550 |
| | Friday | | | | | 98.8 | 317.9 | 61.1 | 2470 |
| | Average | | | | | 116.3 | 303.0 | 79.8 | 2650 |

from 7 a.m. to 7 a.m. and labelled as of the closing date. Therefore, the input of protein in terms of grams of $N_2$ for Monday, August 24 is listed with August 25 urinary nitrogen for comparison. The increase of urinary nitrogen over the usual values is shown in chart II of the previous paper.

Subject 4 and 5 in August show that the urinary nitrogen is 81.7 and 74.4 per cent of the calculated input over the same period. In September, subjects 3, 4 and 5 show that the urinary nitrogen is 89.2, 92.2 and 79.6 per cent respectively of the calculated input over the same period. The accepted value is that about 90 per cent of the nitrogen input is excreted

TABLE 2

| DATE | SUBJECT 3 | | SUBJECT 4 | | SUBJECT 5 | |
|---|---|---|---|---|---|---|
| | Input | Urine | Input | Urine | Input | Urine |
| August 22 | | | | 10.93 | | 7.86 |
| 23 | | | | 10.34 | | 9.68 |
| 24 | | | | 11.13 | | 9.24 |
| 25 | | | 14.28 | 11.48 | 16.04 | 11.00 |
| 26 | | | 15.30 | 12.65 | 16.99 | 12.05 |
| 27 | | | 16.01 | 12.78 | 16.12 | 12.73 |
| 28 | | | 14.67 | 12.84 | 16.20 | 11.76 |
| 29 | | | 14.81 | 11.56 | 16.61 | 13.44 |
| 30 | | | | 10.70 | | 11.65 |
| 31 | | | | 10.90 | | 9.49 |
| September 1 | | | | 10.46 | | 11.56 |
| 2 | | | | 10.55 | | 10.22 |
| 3 | | | | 9.35 | | 7.44 |
| 4 | | | | 8.34 | | 7.09 |
| 5 | | | | 8.96 | | 6.93 |
| 6 | | | | 9.12 | | 8.55 |
| 7 | | | | 10.94 | | 8.58 |
| 8 | | | | 9.50 | | 11.27 |
| 9 | | | | 10.74 | | 8.59 |
| 10 | | 7.66 | | 10.98 | | 6.90 |
| 11 | | 9.58 | | 10.46 | | 8.61 |
| 12 | | | | 10.98 | | 8.86 |
| 13 | | | | 11.27 | | 8.43 |
| 14 | | 8.46 | | 10.84 | | 9.13 |
| 15 | 14.35 | 6.29 | 13.34 | 10.87 | 16.65 | 13.14 |
| 16 | 13.75 | 16.95 | 13.88 | 11.11 | 17.63 | 13.22 |
| 17 | 12.02 | 10.73 | 11.82 | 11.07 | 14.28 | 11.85 |
| 18 | 11.51 | 10.93 | 9.16 | 10.58 | 13.42 | 11.63 |
| 19 | 9.77 | 9.96 | 11.45 | 11.38 | 16.12 | 12.30 |
| 20 | | 7.85 | | 10.14 | | 12.94 |
| 21 | | 7.98 | | 9.74 | | 6.73 |
| 22 | | 5.50 | | 8.94 | | 8.02 |
| 23 | | 7.58 | | 8.77 | | 7.28 |

TABLE 3

*Calories*

| SUBJECT | MONTH | FOUND | 70 KILO | "NORMAL" |
|---|---|---|---|---|
| 4 | August | 2835 | 2790 | 2750 |
| 5 | August | 3030 | 3740 | 2560 |
| 3 | September | 2650 | 3410 | 2530 |
| 4 | September | 2620 | 2580 | 2380 |
| 5 | September | 3280 | 4050 | 2730 |

by the urine while here the average value is 83.4 per cent. This value is open to the criticism that the calculated input may not be the true value. It is hardly to be expected that it is absolutely correct for in respect to the milk alone, the per cent protein used was 3.2, while the city laws say 3.5 per cent which according to the chemist of the dairy was closely adhered to.[1]

This lowered value of 83.4 per cent may be due to altered conditions in the south, such as intestinal putrefaction and perspiration. In the summer the latter has a very appreciable value especially when the nights remain warm and the humidity high. In fact, the perspiration may be one of the contributing causes to the lowered urinary nitrogen in the summer reported by Denis and Borgstrom (3). This will be discussed in a later paper.

The normal diet for a man of 70 kilos doing light work is given by Rubner as 2445 calories while by Atwater 2700 calories. In table 3 are given the calories eaten, calculated to 70 kilos body weight for these experiments. None of these values give a value less than that by Rubner and the two for subject 4 show a value close to that given by Atwater, subjects 3 and 4 in the second run show an excretion of urinary nitrogen very close to the 90 per cent that is the generally accepted value while in both the August and September experiments subject 5 showed a very large discrepancy. This may be partially accounted for by intestinal putrefaction, for subject 5 showed very strong tests for indican, while 3 and 4 were very weak or negative.

In all cases of this work except two days of the September run of subject 4, the subjects felt that they were eating more than normal. This probably is true for in all cases the urinary nitrogen curve showed a higher value than normal. If corrected for this increased value above normal, assuming a mixed diet, it brings a "normal" calorific intake of 2590 calories for 70 kilos body weight. This shows then that the calorific intake in the south does not vary much from the northern climate, but that the protein intake is lower.

Using the values determined by Hafkesbring and Borgstrom (4), the basal calorific requirements for subjects 3, 4 and 5 are 1325, 1400 and 1395 calories per 24 hours, respectively, or an average of 1375 calories for 24 hours. The "normal" calculated value is an average of 2590 calories per day for these experiments, or about twice the "basal" requirements.

[1] The milk purchased was Cloveland's Standard Milk and the analysis given was: 3.6 per cent protein, 4.9 per cent carbohydrate, and 4 per cent fat. These values were not used because all other values were those given by Lusk and the authors did not have the time to check the values and thought it best to use the one set throughout.

SUMMARY

1. A series of experiments was carried on to show the number of calories eaten by a normal individual in a place of warm climate.

2. Results show that none of the values obtained are less than those given by Rubner, and two are close to those given by Atwater; that is, the calorific intake in the south does not vary much from that of the north, but the protein intake is lower.

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# CUTANEOUS EXCRETION OF NITROGENOUS MATERIAL IN NEW ORLEANS

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In 1924, Denis and Borgstrom (5) reported a lower urinary nitrogen output in warm weather than in cold weather, using the 24-hour metabolism reports of medical students. They also report a protein consumption of 73.8 grams, a value distinctly less than the average protein intake (8) for inhabitants of the United States. These facts may be accounted for by 1, lessened protein intake; 2, intestinal putrefaction; 3, perspiration. The first two have already been discussed (3), (4) in previous papers. In this paper, the third will be considered.

It is a well-known fact that the body eliminates nitrogenous substances by way of the skin. F. G. Benedict (1) suggests that the perspiration may account for a considerable portion of the nitrogenous excretion, especially in a tropical climate, even where there is no sensible perspiration. Eijkman (6) found with Malay medical students excretion of 0.761 gram and 1.362 grams respectively. Benedict and Milner (2) report 1.95 and 1.92 grams of nitrogen in perspiration covering three days or about 0.65 gram per day.

The subjects in this work were nos. 3 and 5 (7). Before beginning the experiments, the body was thoroughly scrubbed without using soap. This was followed by a shower bath, and finally the whole body was carefully sponged with cheese cloth and distilled water and then dried. Cotton union suit and cotton stockings, which had been previously washed in distilled water and dried, were then put on. At the end of the experiment, the union suit and stockings were removed and the body washed with distilled water, reserving all the wash water. The stockings and union suit were then carefully washed about ten times with distilled water, wringing all the water out after each extraction. All of the water from the rinsings and from the extraction of the union suit were added together, placed in a large evaporating dish, acidified to prevent loss of ammonia and evaporated. This was filtered through quantitative filter paper to remove extraneous material, epithelial cells, etc., and made up to a definite volume. Therefore, in this work only the water-soluble nitrogenous bodies are considered.

These experiments were carried out in a room 40 by 60 feet, equipped with skylights and six side windows. The temperature and humidity were read at 15-minute intervals, using a Tycos Sling Psychrometer. The analyses for nitrogen were made by the Kjeldahl method.

The results obtained are given in table 1.

The results show that the nitrogenous excretion is roughly proportional to the exercise taken, but differ for each individual. There is a surprising constancy of nitrogenous excretion during the day as is shown by experi-

TABLE 1

| EXPERIMENT | SUBJECT | TEMPERATURE | HUMIDITY | TIME | DURATION | GRAMS $N_2$ IN FOOD* | | | Mgr. | Mgr. $M^2$/hr. |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | a.m. | Noon | p.m. | | |
| 1 | 3 | 89.3 | 71 | p.m. | 2.5 | 3.8 | 7.5 | | 403 | 99.4 |
| 2 | 5 | 89.3 | 71 | p.m. | 2.5 | 5.1 | 6.5 | | 344 | 79.0 |
| 3 | 5 | 90.2 | 73 | p.m. | 2.16 | 4.7 | 5.5 | | 306 | 81.0 |
| 4 | 3 | 89.5 | 71 | p.m. | 3.5 | 3.5 | 5.0 | | 425 | 75.0 |
| 5 | 3 | 90.5 | 62 | a.m. | 3.75 | 3.5 | | | 465 | 76.0 |
| 6 | 5 | 89.5 | 71 | p.m. | 3.0 | 4.8 | 3.8 | | 440 | 84.0 |
| 7 | 3 | 85.0 | 67 | | 24.0 | 3.7 | 2.1 | 4.0 | 900 | 23.0 |
| 8 | 5 | 85.0 | 67 | | 24.0 | 4.7 | 5.0 | 6.4 | 613 | 14.7 |

* Data from metabolism experiments reported by Borgstrom, Bost and Hafkesbring.

*Notes on Experiments*

I and II. Monday. Exercised alternately with springs and running in a slow trot.

III. Tuesday. Exercised 5 minutes. Rested 10 minutes. Alternately for first hour. Remainder of time sat in sun under skylight.

IV. Wednesday. Walked streets in business district, shopping. Subject dressed in linen suit, at no time excessively warm.

V. Thursday. Same as IV, except in a.m.

VI. Wednesday. First hour, exercised alternately 15 minutes with springs, rest 5 minutes. Second hour, alternately ran slowly 15 minutes, rest 5 minutes. Next 30 minutes did calisthenics; last 30 minutes, sat in sun. Work harder than in III.

VII. Friday. Walked 10 blocks morning and evening. During day did work of office type.

VIII. Friday. Same as VII, except that subject walked 20 blocks morning and evening.

ments IV and V. The food intake does not seem to affect the value found for in experiments V, 3½ grams of nitrogen had been ingested in the last 12 hours, while with experiment IV, it was 8½ grams, yet the excretion per square meter body surface per hour was practically the same.

Experiments VII and VIII show that there is nitrogenous excretion even when there is no appreciable sensible perspiration for in these experiments no attempt was made to cause excessive perspiration. Experiment VII shows that the perspiration accounted for 9.3 per cent of nitrogen input, a

value surprisingly high. Even then the excretion for subject 3 was only 22 mgm. per square meter per hour, while in experiments IV and V it was 99, 75 and 76 mgm. per square meter per hour, values four and three times higher than for the 24-hour period. For experiment VIII the cutaneous excretion was 3.8 per cent of the input for subject 5. This value is not as high as for subject 3, but comparing experiments II, III, VI and VIII the cutaneous nitrogenous excretion was 79, 81, 84 and 14.7 mgm. per square meter per hour. The excretion for experiments II, III and VI is five to six times that for experiment VIII, so if sensible perspiration occurred, the loss of nitrogen through the skin would be very appreciable and not to be neglected.

With perspiration values as high as these it is not at all surprising that part of the drop in summer (3), (5) in the urinary nitrogen may be due to perspiration. The difference between summer and winter values (5) of the urinary nitrogen is 1.35 grams. The highest value of perspiration for 24 hours is 0.9 gram or nearly 70 per cent of the reported difference. The perspiration can then readily account for a large part of the decrease in urinary nitrogen in the summer, as these values of nitrogenous bodies in the perspiration if anything are low, due to evaporation of ammonia from the skin, and some decomposition of urea.

## SUMMARY

1. Experiments show that induced perspiration and perspiration from walking runs about 75 mgm. nitrogen per square meter body surface.

2. The protein content of food eaten in the 12 hours previous to the experiments does not seem to alter the amount of nitrogenous bodies excreted per square meter body surface to any appreciable extent.

3. The morning and afternoon parts of the day show a much larger nitrogenous excretion per square meter per hour than does the 24-hour period.

4. The relationship of the cutaneous nitrogen excretion to the total input is discussed, especially in reference to summer weather.

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# EFFECT OF INCREASED DIET ON BASAL METABOLISM IN NEW ORLEANS

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There is a question whether the basal metabolism is a constant value or varies from day to day (6). Hafkesbring and Collett (5) give the day to day variation in basal metabolism of women in New Orleans, but with the information given by Hafkesbring and Borgstrom (4), this variation might be reduced to a smaller value if the minimum is used. With the basal metabolism nearly constant on a mixed diet, there is a question whether a change in the dietary will cause a shift in this value.

There has been a large amount of work done on the effect of ingested foodstuffs on the metabolism, but most of this work covers only the first six to eight hours when digestion is most active. It has been well established that the influence on metabolism is most marked with proteins and least with fats. As it is laborious, and for us almost impossible, to determine the amount of fat, carbohydrate and protein in the diet, the alternative was done; that is, the amount of the urinary nitrogen was checked as just one of the factors influencing the metabolism. In four series of five days each, a weighed diet was used.

The method used for determining the basal metabolism is described by Hafkesbring and Borgstrom (4). The subjects were 3, 4, 5, 6 and 9 of the same paper. Subject 10 was a woman, 23 years old, 152 cm. tall, and weighing 44 kilograms.

The determinations were made about the same time after the last meal. Where a series is made day after day, as with subject 4, it was rather difficult for the subject to eat enough food to satisfy hunger in the six to seven hours available.

The results found are given in table 1. The oxygen consumption and urinary nitrogen are plotted on chart I for subjects 3, 5, 6, 9 and 10, and on chart II for subject 4. This last chart gives the results of a series where the minimum, when possible, was determined as well as the 14.5 hour value.

The values for the urinary nitrogen for subjects 3 and 4 in reference to the weekly values are given in chart II and table 3 by Borgstrom and Bost (2). The calorific value of the dietary of subjects 3, 4 and 5 as well as

urinary nitrogen are given by Borgstrom, Bost and Hafkesbring (3) in table 1 for the period of the experiment as well as the intervening period.

TABLE 1

| DATE | °C. | $N_2$ | HOURS | PULSE | VENTILATION | $CO_2$ | $O_2$ | R.Q. | $O_2$ cc./MIN. | CAL. $M_2$/hr. |
|---|---|---|---|---|---|---|---|---|---|---|
| **Subject 3** | | | | | | | | | | |
| September 1925 | | | | | | | | | | |
| 14 | 28.53 | 8.46 | 14.15 | 78 | 4.29 | 3.43 | 4.83 | 0.710 | 207 | 35.92 |
| 15 | 28.89 | 6.29 | 14.15 | 80 | 4.40 | 3.60 | 4.66 | 0.772 | 205 | 36.23 |
| 16 | 28.89 | 16.95 | 14.25 | 84 | 4.45 | 3.54 | 4.83 | 0.733 | 215 | 37.54 |
| 17 | 30.00 | 10.73 | 14.15 | 78 | 4.43 | 3.18 | 3.61 | 0.882 | 160 | 29.02 |
| 18 | 30.00 | 10.93 | 14.00 | 82 | 4.38 | 3.65 | 4.96 | 0.736 | 218 | 38.10 |
| 19 | 30.00 | 9.96 | 13.90 | 78 | 4.68 | 3.38 | 4.92 | 0.687 | 230 | 39.90 |
| 20 | 30.00 | 7.85 | 14.40 | 80 | 4.72 | 3.02 | 3.70 | 0.816 | 175 | 31.20 |
| 21 | 30.00 | 7.98 | 13.60 | 79 | 4.63 | 3.38 | 3.68 | 0.918 | 170 | 31.11 |
| 22 | 31.11 | 5.50 | 13.45 | 78 | 5.20 | 3.28 | 4.30 | 0.763 | 224 | 39.38 |
| 23 | 30.00 | 7.58 | 13.40 | 76 | 4.73 | 3.67 | 4.52 | 0.812 | 214 | 38.12 |
| October | | | | | | | | | | |
| 10 | 15.56 | 8.74 | 13.85 | 80 | 4.35 | 3.46 | 4.38 | 0.790 | 191 | 33.82 |
| 11 | 20.00 | 9.87 | 14.05 | 78 | 4.39 | 3.58 | 4.57 | 0.784 | 201 | 35.55 |
| 12 | 26.67 | 7.97 | 13.90 | 80 | 4.66 | 3.44 | 4.58 | 0.751 | 213 | 37.35 |
| 13 | 27.78 | 9.55 | 14.10 | 84 | 4.91 | 3.50 | 3.84 | 0.912 | 189 | 34.52 |
| 14 | 25.56 | 11.96 | 14.05 | 83 | 4.30 | 3.73 | 4.37 | 0.852 | 188 | 33.85 |
| 15 | 24.44 | 6.20 | 14.10 | 80 | 4.68 | 3.46 | 4.32 | 0.802 | 202 | 35.89 |
| 16 | 26.67 | 7.51 | 14.00 | 84 | 4.54 | 3.45 | 3.82 | 0.903 | 173 | 31.55 |
| 17 | 25.56 | 11.87 | 14.05 | 86 | 4.61 | 3.63 | 3.69 | 0.984 | 170 | 31.62 |
| 18 | 25.56 | 6.61 | 13.90 | 77 | 4.70 | 3.14 | 4.18 | 0.751 | 197 | 34.58 |
| 18 | 25.56 | 6.61 | 23.60 | 82 | 4.80 | 3.06 | 4.03 | 0.760 | 193 | 33.92 |
| 18 | 25.56 | 6.61 | 27.95 | 80 | 4.38 | 3.40 | 4.61 | 0.738 | 201 | 35.11 |
| 19 | 17.78 | 11.06 | 13.95 | 77 | 4.60 | 3.19 | 3.82 | 0.835 | 176 | 31.55 |
| **Subject 4** | | | | | | | | | | |
| August | | | | | | | | | | |
| 24 | 25.56 | 11.13 | 15.40 | 78 | 5.15 | 3.09 | 4.20 | 0.736 | 216 | 33.62 |
| 25 | 24.44 | 11.48 | 15.40 | 78 | 5.49 | 3.34 | 3.80 | 0.882 | 208 | 33.05 |
| 26 | 27.78 | 12.65 | 15.20 | 80 | 5.65 | 3.43 | 4.15 | 0.825 | 234 | 37.20 |
| 27 | 28.89 | 12.78 | 15.40 | 76 | 5.21 | 3.29 | 4.30 | 0.766 | 224 | 35.10 |
| 28 | 29.44 | 12.84 | 15.40 | 79 | 5.43 | 3.31 | 4.46 | 0.742 | 242 | 37.65 |
| 29 | 26.67 | 11.56 | 15.40 | 80 | 5.23 | 3.33 | 4.33 | 0.769 | 227 | 35.58 |
| 30 | | | | | | | | | | |
| 31 | 25.56 | 10.90 | 15.30 | 78 | 5.22 | 3.24 | 3.91 | 0.832 | 205 | 32.65 |
| January | | | | | | | | | | |
| 12 | 9.48 | 7.40 | 13.10 | 80 | 5.34 | 3.16 | 4.31 | 0.733 | 230 | 35.69 |
| 12 | | | 14.30 | 76 | 5.60 | 3.19 | 4.09 | 0.780 | 229 | 36.00 |
| 12 | | | 15.50 | 78 | 5.22 | 3.12 | 3.85 | 0.811 | 201 | 31.82 |
| 12 | | | 18.30 | 76 | 5.55 | 3.26 | 3.88 | 0.841 | 215 | 34.32 |
| 13 | 5.56 | 8.62 | 14.30 | 78 | 5.79 | 3.16 | 4.07 | 0.777 | 236 | 37.08 |

TABLE 1—*Continued*

| DATE | °C. | $N_2$ | HOURS | PULSE | VENTILATION | $CO_2$ | $O_2$ | R.Q. | $O_2$ cc./MIN. | CAL. $M_2$/hr. |
|---|---|---|---|---|---|---|---|---|---|---|
| Subject 4—Continued | | | | | | | | | | |
| January 1926 | | | | | | | | | | |
| 13 | | | 15.60 | 80 | 5.92 | 3.08 | 3.27 | 0.942 | 193 | 31.60 |
| 13 | | | 17 20 | 80 | 5.40 | 3.12 | 4.08 | 0.765 | 220 | 34.45 |
| 13 | | | 18.60 | 78 | 5.81 | 3.07 | 4.11 | 0.747 | 231 | 36.00 |
| 14 | 6.67 | 7.77 | 13.80 | 78 | 6.13 | 3.18 | 3.76 | 0.846 | 230 | 36.80 |
| 14 | | | 15.60 | 76 | 5.58 | 3.15 | 3.77 | 0.835 | 211 | 33.61 |
| 14 | | | 17.50 | 76 | 5.28 | 3.27 | 4.18 | 0.782 | 221 | 34.75 |
| 15 | 9.48 | 9.70 | 14.40 | 80 | 5.81 | 3.34 | 3.76 | 0.888 | 219 | 35.38 |
| 16 | 11.11 | 9.77 | 10.00 | 84 | 6.02 | 3.31 | 4.66 | 0.710 | 282 | 43.55 |
| 16 | 11.11 | 9.77 | 12.33 | 80 | 5.86 | 3.42 | 3.32 | 1.030 | 195 | |
| 16 | | | 14.70 | 80 | 5.44 | 3.17 | 2.50 | 1.269 | 139 | |
| 16 | | | 17.00 | 77 | 5.76 | 3.11 | 3.58 | 0.868 | 206 | 33.11 |
| 17 | | | | | | | | | | |
| 18 | 11.67 | 11.82 | 14.30 | 79 | 5.64 | 3.44 | 3.13 | 1.099 | 171 | |
| 19 | 12.78 | 13.11 | 14.40 | 82 | 5.96 | 3.36 | 3.41 | 0.985 | 203 | 33.60 |
| 19 | | | 15.75 | 80 | 5.52 | 3.27 | 3.95 | 0.828 | 218 | 34.68 |
| 19 | | | 18.20 | 82 | 6.10 | 3.29 | 3.77 | 0.872 | 230 | 37.01 |
| 20 | 15.04 | 12.48 | 14.30 | 82 | 5.92 | 3.48 | 3.90 | 0.892 | 231 | 37.35 |
| 20 | | | 17.20 | 80 | 5.81 | 3.17 | 3.70 | 0.857 | 215 | 34.45 |
| 20 | | | 18.50 | 78 | 5.78 | 3.29 | 3.79 | 0.868 | 219 | 35.40 |
| 21 | 21.11 | 12.44 | 14.60 | 78 | 5.95 | 3.26 | 3.16 | 1.032 | 188 | |
| 21 | | | 16.20 | 78 | 5.89 | 3.39 | 3.51 | 0.968 | 207 | 34.41 |
| 21 | | | 18.60 | 80 | 6.16 | 3.33 | 3.53 | 0.943 | 217 | 35.55 |
| 22 | 3.33 | 15.22 | 14.30 | 80 | 6.04 | 3.42 | 2.76 | 1.238 | 167 | |
| 22 | | | 15.80 | 78 | 6.10 | 3.27 | 3.48 | 0.940 | 212 | 34.68 |
| 22 | | | 15.90 | 78 | 5.79 | 3.18 | 3.53 | 0.901 | 205 | 33.22 |
| 22 | | | 17.60 | 80 | 6.73 | 3.30 | 3.42 | 0.964 | 230 | 37.88 |
| 23 | 1.67 | 14.31 | 15.70 | 80 | 5.62 | 3.32 | 3.66 | 0.907 | 206 | 33.45 |
| 23 | | | 17.40 | 74 | 5.64 | 3.34 | 3.34 | 1.000 | 188 | 31.38 |
| 23 | | | 17.60 | 74 | 5.86 | 3.34 | 3.28 | 1.018 | 192 | |
| 24 | 1.67 | 11.90 | 14.65 | 76 | 5.85 | 3.50 | 3.56 | 0.983 | 208 | 34.40 |
| 24 | | | 15.75 | 76 | 5.79 | 3.42 | 3.75 | 0.912 | 217 | 35.27 |
| 25 | 4.44 | 12.71 | 14.20 | 78 | 6.10 | 3.38 | 2.94 | | 179 | |
| 25 | | | 14.30 | 78 | 5.73 | 3.46 | 3.17 | | 182 | |
| 26 | 3.89 | 16.26 | 14.30 | 80 | 5.30 | 3.37 | 3.68 | 0.916 | 195 | 31.72 |
| 26 | | | 15.60 | 88 | 5.47 | 3.23 | 3.52 | 0.918 | 193 | 31.40 |
| 26 | | | 17.70 | 80 | 5.95 | 3.40 | 3.70 | 0.919 | 222 | 36.12 |
| 27 | 9.48 | 9.68 | 14.50 | 76 | 5.48 | 3.37 | 4.08 | 0.826 | 224 | 35.62 |
| 27 | | | 15.80 | 75 | 5.65 | 3.48 | 3.73 | 0.933 | 211 | 34.45 |
| 27 | | | 17.70 | 79 | 5.64 | 3.44 | 4.07 | 0.846 | 230 | 36.76 |
| 28 | 10.00 | 10.84 | 13.00 | 80 | 5.85 | 3.44 | 3.56 | 0.966 | 209 | 34.42 |
| 28 | | | 14.00 | 78 | 5.54 | 3.47 | 3.56 | 0.974 | 197 | 32.50 |
| 28 | | | 15.00 | 78 | 5.39 | 3.37 | 3.91 | 0.862 | 211 | 33.85 |
| 28 | | | 16.00 | 78 | 6.22 | 3.43 | 3.55 | 0.966 | 221 | 36.39 |
| 28 | | | 17.00 | 80 | 5.52 | 3.24 | 4.21 | 0.770 | 232 | 36.39 |

TABLE 1—*Continued*

| DATE | °C. | $N_2$ | HOURS | PULSE | VENTILATION | $CO_2$ | $O_2$ | R.Q. | $O_2$ CC./MIN. | CAL. $M_2$/hr. |
|---|---|---|---|---|---|---|---|---|---|---|
| **Subject 4—Continued** | | | | | | | | | | |
| January 1926 | | | | | | | | | | |
| 28 | | | 18.00 | 80 | 5.55 | 3.38 | 4.06 | 0.832 | 226 | 36.00 |
| 29 | 10.56 | 10.52 | 14.00 | 78 | 5.78 | 3.34 | 3.28 | 1.015 | 190 | |
| 29 | | | 15.50 | 76 | 5.87 | 3.40 | 3.69 | 0.920 | 217 | 35.32 |
| 29 | | | 15.60 | 76 | 6.08 | 3.39 | 3.78 | 0.897 | 230 | 37.22 |
| 30 | 9.48 | 9.44 | 14.00 | 77 | 5.60 | 3.43 | 3.77 | 0.910 | 211 | 34.25 |
| February | | | | | | | | | | |
| 1 | 12.22 | 8.70 | 14.00 | 79 | 5.42 | 3.40 | 4.49 | 0.758 | 243 | 38.00 |
| 1 | | | 14.10 | 79 | 5.78 | 3.49 | 4.07 | 0.858 | 235 | 37.70 |
| 1 | | | 16.50 | 78 | 5.52 | 3.24 | 3.57 | 0.908 | 201 | 32.62 |
| 2 | 12.22 | 8.35 | 14.20 | 78 | 5.76 | 3.27 | 3.94 | 0.830 | 227 | 36.14 |
| 2 | | | 16.00 | 80 | 5.92 | 3.12 | 3.17 | 0.984 | 188 | 31.09 |
| **Subject 5** | | | | | | | | | | |
| August | | | | | | | | | | |
| 24 | 25.56 | 9.24 | 16.00 | 80 | 6.10 | 2.99 | 3.42 | 0.874 | 209 | 35.15 |
| 25 | 24.42 | 11.00 | 16.00 | 79 | 5.73 | 2.53 | 4.65 | 0.544 | 267 | |
| 26 | 27.78 | 12.22 | 16.00 | 78 | 6.18 | 2.94 | 4.65 | 0.632 | 287 | |
| 27 | 28.89 | 12.61 | 16.25 | 76 | 6.02 | 3.24 | 3.43 | 0.944 | 207 | 35.45 |
| 28 | 29.44 | 11.65 | 15.95 | 74 | 6.34 | 2.77 | 3.97 | 0.698 | 252 | 40.60 |
| 29 | 26.67 | 13.31 | 16.05 | 75 | 6.68 | 3.10 | 4.20 | 0.738 | 280 | 45.50 |
| 30 | | 11.65 | | | | | | | | |
| 31 | 25.56 | 9.49 | 16.00 | 76 | 6.18 | 3.18 | 4.08 | 0.780 | 252 | 41.40 |
| September | | | | | | | | | | |
| 1 | 25.56 | 11.56 | 15.90 | 78 | 6.14 | 3.07 | 3.99 | 0.770 | 245 | 40.12 |
| 14 | 28.53 | 9.13 | 15.00 | | 6.07 | 2.65 | 3.15 | 0.841 | 191 | 31.85 |
| 15 | 28.89 | 13.14 | 14.85 | 80 | 5.93 | 3.14 | 4.31 | 0.728 | 255 | 41.33 |
| 16 | 28.89 | 13.20 | 15.15 | 78 | 5.90 | 2.77 | 3.24 | 0.855 | 191 | 32.00 |
| 17 | 30.00 | 11.85 | 14.95 | 78 | 6.08 | 3.19 | 3.43 | 0.930 | 209 | 35.62 |
| 18 | 30.00 | 11.63 | 14.70 | 76 | 6.36 | 3.16 | 4.71 | 0.671 | 299 | |
| 19 | 30.00 | 12.30 | 14.70 | 80 | 5.97 | 3.32 | 5.17 | 0.642 | 300 | |
| 21 | 30.00 | 6.73 | 14.50 | 82 | 6.01 | 3.15 | 3.70 | 0.852 | 222 | 37.11 |
| 22 | 31.11 | 8.02 | 14.00 | 79 | 5.88 | 3.24 | 5.07 | 0.639 | 298 | 48.00 |
| 23 | 30.00 | 7.27 | 13.90 | 80 | 5.70 | 3.35 | 4.37 | 0.766 | 249 | 40.75 |
| **Subject 6** | | | | | | | | | | |
| October 1925 | | | | | | | | | | |
| 10 | 15.56 | 10.91 | 13.50 | 58 | 5.65 | 2.93 | 3.64 | 0.804 | 205 | 32.38 |
| 11 | 20.00 | 8.44 | 14.00 | 54 | 5.13 | 3.16 | 3.88 | 0.814 | 199 | 31.55 |
| 12 | 26.67 | 9.53 | 13.40 | 57 | 5.36 | 3.03 | 3.93 | 0.771 | 211 | 33.02 |
| 13 | 27.78 | 13.63 | 13.75 | 63 | 5.70 | 2.74 | 3.22 | 0.850 | 184 | 29.40 |
| 14 | 25.56 | 14.81 | 13.50 | 62 | 5.70 | 3.13 | 3.36 | 0.932 | 195 | 31.82 |
| 15 | 24.44 | 18.31 | 13.70 | 66 | 6.40 | 2.91 | 3.07 | 0.948 | 196 | 32.09 |

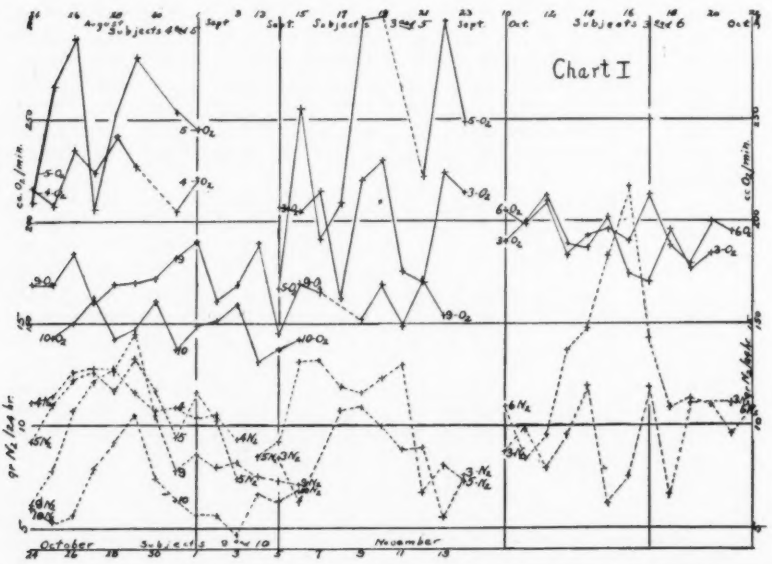
TABLE 1—*Continued*

| DATE | °C. | $N_2$ | HOURS | PULSE | VENTILATION | $CO_2$ | $O_2$ | R.Q. | $O_2$ cc./MIN. | CAL. $M_2$/hr. |
|---|---|---|---|---|---|---|---|---|---|---|
| **Subject 6—Continued** | | | | | | | | | | |
| October 1925 | | | | | | | | | | |
| 16 | 26.67 | 21.73 | 13.50 | 62 | 6.13 | 2.86 | 3.11 | 0.920 | 191 | 31.05 |
| 16 | | | 18.75 | 67 | 5.98 | 3.00 | 3.49 | 0 859 | 209 | 33 48 |
| 16 | | | 22.85 | 66 | 6.35 | 2.73 | 3.97 | 0.688 | 252 | |
| 17 | 25.56 | 14.38 | 13.55 | 66 | 5.98 | 2.93 | 3.55 | 0.826 | 212 | 33.67 |
| 18 | 23.89 | 10.91 | 14.45 | 60 | 5.84 | 3.11 | 3.22 | 0.966 | 188 | 30.75 |
| 19 | 17.78 | 11.32 | 13.45 | 57 | 5.86 | 2.95 | 3.05 | 0.967 | 179 | 29.43 |
| 20 | 13.33 | 11.02 | 13.50 | 70 | 5.83 | 3.03 | 3.43 | 0.883 | 200 | 32.20 |
| 21 | 15.56 | 9.66 | 13.50 | 60 | 6.11 | 3.19 | 3.19 | 1.000 | 195 | 32.32 |
| **Subject 9** | | | | | | | | | | |
| October | | | | | | | | | | |
| 24 | 26.67 | 6.00 | 13.60 | 80 | 4.46 | 3.02 | 3.88 | 0.778 | 169 | 32.87 |
| 25 | 21.11 | 7.78 | 14.00 | 76 | 4.51 | 3.16 | 3.70 | 0.854 | 169 | 33.50 |
| 26 | 15.56 | 10.75 | 13.15 | 81 | 4.89 | 3.61 | 3.78 | 0.690 | 184 | 35.10 |
| 27 | 16.67 | 12.10 | 13.05 | 70 | 4.60 | 2.90 | 3.48 | 0.833 | 162 | 31.95 |
| 28 | 16.67 | 12.69 | 13.10 | 76 | 4.72 | 2.81 | 3.60 | 0.780 | 168 | 32.68 |
| 29 | 12.22 | 14.52 | 13.15 | 80 | 4.78 | 2.66 | 3.52 | 0.748 | 170 | 32.80 |
| 30 | 14.44 | 10.49 | 13.30 | 78 | 4.75 | 2.79 | 3.67 | 0.760 | 172 | 33.30 |
| 31 | 11.11 | 7.76 | 13.35 | 76 | 5.10 | 2.89 | 3.60 | 0.803 | 184 | 36.00 |
| November | | | | | | | | | | |
| 1 | 13.33 | 8.55 | 14.25 | 78 | 4.55 | 2.81 | 4.15 | 0.678 | 190 | 36.25 |
| 2 | 16.67 | 7.95 | 13.25 | 80 | 4.70 | 2.98 | 3.43 | 0.868 | 161 | 32.22 |
| 3 | 18.89 | 8.18 | 13.15 | 74 | 4.54 | 2.73 | 3.72 | 0.734 | 169 | 32.47 |
| 4 | 21.11 | 7.50 | 13.15 | 78 | 4.89 | 2.78 | 3.82 | 0.728 | 188 | 36.10 |
| 5 | 18.89 | 7.30 | 13.25 | 72 | 4.96 | 2.76 | 2.96 | 0.932 | 145 | 29.30 |
| 6 | 20.00 | 7.10 | 13.20 | 74 | 4.78 | 2.81 | 3.55 | 0.791 | 169 | 32.98 |
| 7 | 23.89 | | 13.70 | 80 | 4.67 | 2.85 | 3.43 | 0.808 | 165 | 32.32 |
| 8 | 16.11 | | | | | | | | | |
| 9 | 12.22 | | 13.05 | 74 | 4.48 | 2.86 | 3.64 | 0.786 | 162 | 31.58 |
| 10 | 15.56 | | 13.05 | 74 | 4.49 | 2.94 | 3.76 | 0.782 | 169 | 32.88 |
| 11 | 18.89 | | 13.00 | 76 | 4.54 | 2.90 | 3.30 | 0.878 | 149 | 29.70 |
| 12 | 20.00 | | 13.10 | 73 | 4.14 | 3.14 | 4.13 | 0.760 | 171 | 33.08 |
| 13 | 18.89 | | 13.10 | 76 | 4.10 | 3.19 | 3.77 | 0.846 | 154 | 30.48 |
| **Subject 10** | | | | | | | | | | |
| October 1925 | | | | | | | | | | |
| 25 | 21.11 | 5.25 | 14.00 | 76 | 4.58 | 2.51 | 3.10 | 0.810 | 142 | 30.00 |
| 26 | 18.89 | 5.62 | 13.15 | 76 | 4.83 | 2.78 | 3.11 | 0.894 | 150 | 32.35 |
| 27 | 16.67 | 7.90 | 13.15 | 80 | 4.91 | 2.63 | 3.33 | 0.790 | 163 | 34.21 |
| 28 | 16.67 | 9.18 | 13.15 | 72 | 4.92 | 2.65 | 2.89 | 0.917 | 142 | 30.80 |
| 29 | 12.32 | 10.43 | 13.15 | 80 | 4.79 | 2.59 | 3.09 | 0.838 | 147 | 31.24 |
| 30 | 14.44 | 7.41 | 13.45 | 78 | 4.41 | 2.88 | 3 59 | 0.802 | 161 | 33.92 |
| 31 | 11.11 | 6.38 | 13.50 | 88 | 4.87 | 2.74 | 3.12 | 0.878 | 152 | 32.62 |

TABLE 1—*Concluded*

| DATE | °C. | $N_2$ | HOURS | PULSE | VENTILATION | $CO_2$ | $O_2$ | R.Q. | $O_2$ cc./MIN. | CAL. $M_2$/hr. |
|---|---|---|---|---|---|---|---|---|---|---|
| Subject 10—Continued | | | | | | | | | | |
| November 1925 | | | | | | | | | | |
| 1 | 13.33 | 5.68 | 14.25 | 72 | 5.18 | 2.45 | 2.97 | 0.825 | 149 | 31.58 |
| 2 | 16.67 | 5.65 | 13.15 | 72 | 5.27 | 2.60 | 2.93 | 0.888 | 151 | 32.52 |
| 3 | 18.89 | 4.70 | 13.25 | 72 | 4.89 | 2.71 | 3.20 | 0.847 | 159 | 33.89 |
| 4 | 21.11 | 4.62 | 13.25 | 72 | 4.94 | 2.64 | 2.58 | 1.024 | 128 | |
| 5 | 18.89 | 6.29 | 13.35 | 72 | 5.51 | 2.48 | 2.57 | 0.965 | 137 | 30.08 |
| 6 | 20.00 | 6.82 | 13.15 | 68 | 5.47 | 2.54 | 2.60 | 0.979 | 142 | 31.28 |

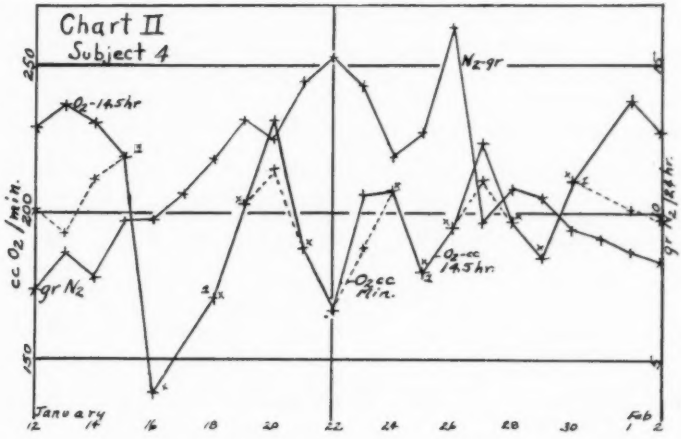
In above table, the values for the calories per square meter per hour are omitted when the R.Q. is above 1.0 and below 0.707. Boothby and Sandiford (1) neglect the low $O_2$ values and calculate calorific output from the $CO_2$, assuming an R.Q. of 1.0 when such readings as these are found.




These results show that when there is an increased food intake as expressed by increased urinary nitrogen, there is a large fluctuation in the basal metabolism as shown by readings taken at a definite time from the last meal. The first thing that occurs is a marked decrease in the oxygen consumption with a fairly constant value for the $CO_2$ output. This occurs within 48 hours after the changed food intake has begun. If the

food intake is greatly increased as with subject 4 and is kept as constant as possible without a weighed diet, this lowered $O_2$ consumption comes back to the normal value (see chart II) with an R. Q. about normal. But if the intake is again increased, the same phenomenon occurs. In fact, it seems that the body can become adjusted to a different level of food intake, but with each increase equilibrium is disturbed and a drop in basal metabolism occurs. When the subject goes back to the ordinary mixed diet with the normal urinary nitrogen, the basal metabolism becomes fairly constant as shown by subjects 4 and 9 in charts I and II, and using the minimum value, as for 4, surprisingly so.

The first drop may be due to several causes. First, with greater protein input, there is a corresponding sulfate and phosphate input. This, with the fatty acids, both ingested and formed by splitting of the proteins,




causes a displacement of the carbonate equilibrium, a freeing of $CO_2$. This may be one of the factors but not the main one, for the $CO_2$ curve shows very slight increase in comparison to the decrease of the $O_2$ curve.

Another cause is probably that the body does not oxidize the normal materials supplied. With excess food, first there is probably a conversion of the amino acids into fatty acids, either by de-carboxylation and oxidation of resulting alcohols, or by beta-oxidation, in the first case, with an R. Q. of 1.0, and in the second, 0.80. But there may be also an excess of carbohydrate eaten and a transformation of these into fats. Starling (7) states that in this case, there may be a setting free of oxygen to care for other purposes. In such a case, there will appear the high R. Q. which may well account for the decreased $O_2$ consumption as shown by these experiments.

The question that arises is whether this is just a characteristic of the warmer climate with its lower basal metabolism as shown by Hafkesbring and Borgstrom (4), or general. If just of local concern, it makes the determination of basal metabolism in warm climates that much more difficult, in that the subject or patient must be kept on the diet to which he is accustomed. If in a hospital, under a diet, this may be different than the normal, with resulting abnormal variation which can hardly be accounted for by experimental errors. Table 2 gives the extremes when the basal metabolism is taken at a definite time. Subject 5 shows a fluctuation from the extremes of −22.0 to +22 per cent, with a food intake of 3280 calories, while with food intake of 3030 calories, the fluctuation was −15.8 to +12.6 per cent, a value distinctly lower with lessened food intake. The others show a corresponding variation with increased food intake.

TABLE 2

| SUBJECT | DATE | DUBOIS | LOW PER CENT | HIGH PER CENT | AVERAGE PER CENT |
|---|---|---|---|---|---|
| 3 | September | 220 | 160−27.2 | 230 +4.5 | 202 −8.2 |
| | October | 220 | 170−22.7 | 213 −3.2 | 191−18.6 |
| 4 | August | 247 | 205−17.0 | 242 −2.0 | 222−10.1 |
| 5 | August | 246 | 207−15.8 | 287+12.6 | 251 +2.0 |
| | September | 246 | 191−22.3 | 300+21.9 | 246 |
| 6 | October | 247 | 165−29.1 | 212−14.1 | 199−19.4 |
| 9 | October | 187 | 145−22.4 | 190+1.6 | 169−9.5 |
| | November | | | | |
| 10 | October | 174 | 128−26.4 | 163 −6.3 | 148−14.9 |
| | November | | | | |

With fluctuations as large as these and with values lower than northern standards, it seems as though the question in warm climates is far from settled. In the south, where there is a long, hot, humid summer, with very few cool days, and with a resulting adjustment of the body to these conditions, many questions of food intake, basal metabolism, etc., need to be investigated to see if all the factors hold the same as in the temperate climates, where most of the work has been done so far. It is questionable if the data on a subject who has just come to a place are as valuable as on one who has become fully adjusted to the locality. The whole subject of how the body works as a machine needs investigation, for, with lowered protein intake, lowered basal metabolism, and a question if the calorific intake is the same, one wonders if the body has the same reserve to combat

disease as it has in colder climates, or whether the effect comes in some other way, such as efficiency of labor. We feel that the processes of metabolism such as digestion, absorption, oxidation, etc., go on here the same as in colder climates, but if the energy input and output are the same, if the machine responds the same to extra fuel—these and other questions are yet to be solved. The authors hope the information given in this and previous papers will help some one with the work, for we feel that this is only a beginning in a study which may bring very beneficial information of a theoretical as well as clinical nature.

## SUMMARY

1. With increased food intake as shown by increased urinary nitrogen, there is a large fluctuation in the basal metabolism, when taken at a definite time from the last meal.

2. The possible causes of this fluctuation are discussed.

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